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(54) Title: HUMAN GALACTOKINASE GENE

(57) Abstract

This invention relates to human galactokinase and the identification of galactokinase mutations, a missense and nonsense, as well as isolated nucleic acids encoding same, recombinant host cell transformed with DNA encoding such proteins and to uses of the expressed proteins and nucleic acid sequences in therapeutic and diagnostic applications.

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Human Galactokinase Gene

This invention was made in part with government support under EY-09404 awarded by the National Institutes of Health. The U.S. Government has certain rights in the invention.

Cross-Reference to Related Applications:

This application is a continuation in part of Serial No. PCT/US94/10825, filed 23 September 1994.

Field of the Invention:

This invention relates to human galactokinase and the identification of galactokinase mutations, a missense and nonsense, as well as isolated nucleic acids encoding same, recombinant host cell transformed with DNA encoding such proteins and to uses of the expressed proteins and nucleic acid sequences in therapeutic and diagnostic applications.

Background of the Invention:

There are numerous inherited human metabolic disorders, most of which are recessive. Many have devastating effects that may include a combination of several clinical features, such as severe mental retardation, impairment of the peripheral nervous system, blindness, hearing deficiency and organomegaly. Most of the disorders are rare. However, the majority of such disorders cannot be treated by drugs.

Galactokinase deficiency is one of three known forms of galactosemia. The other forms are galactose-1-phosphate uridyltransferase deficiency and UDP-galactose-4-epimerase deficiency. All three enzymes are involved in galactose metabolism, i.e., the conversion of galactose to glucose in the body. Galactokinase deficiency is inherited as an autosomal recessive trait with a heterozygote frequency estimated to be 0.2% in the general population (see, e.g., Levy et al., J. Pediatr., 92:871-877 (1978)). Patients with homozygous galactokinase deficiency usually become symptomatic in the early infantile period showing galactosemia, galactosura, increased galactitol levels, cataracts and in a few cases, mental retardation (Segal et al., J. Pediatr., 95:750-752 (1979)). These symptoms usually improve dramatically with the administration of a galactose free diet. Heterozygotes for galactokinase deficiency are prone to presenile cataracts with the

onset during 20-50 years of age (Stambolian et al., Invest. Ophthal. Vis. Sci., 27:429-433 (1986)).

Galactokinase activity has been found in a variety of mammalian tissues, including liver, kidney, brain, lens, placenta, erythrocytes and leukocytes. While the protein has been purified from *E. coli*, the purification of the protein from mammalian tissues has proven difficult due to its low cellular concentration. In addition, the molecular basis of galactokinase deficiency is unknown.

This invention provides a human galactokinase gene. The DNAs of this invention, such as the specific sequences disclosed herein, are useful in that they encode the genetic information required for expression of this protein. Additionally, the sequences may be used as probes in order to isolate and identify additional members, of the family, type and/or subtype as well mutations which may form the basis of galactokinase deficiency which may be characterized by site-specific mutations or by atypical expression of the galactokinase gene. The galactokinase gene is also useful as a diagnostic agent to identify mutant galactokinase proteins or as a therapeutic agent via gene therapy.

The first clinical trials of gene therapy began in 1990. Since that time, more than 70 clinical trial protocols have been reviewed and approved by a regulatory authority such as the NIH's Recombinant Advisory Committee (RAC), see, e.g., Anderson, W. F., Human Gene Therapy, 5:281-282 (1994). The therapeutic treatment of diseases and disorders by gene therapy involves the transfer and stable insertion of new genetic information into cells. The correction of a genetic defect by re-introduction of the normal allele of a gene has hence demonstrated that this concept is clinically feasible (see, e.g., Rosenberg et al., New Eng. J. Med., 323: 570 (1990)).

These and additional uses for the reagents described herein will become apparent to those of ordinary skill in the art upon reading this specification.

Summary of the Invention:

This invention provides isolated nucleic acid molecules encoding human galactokinase, as well as nucleic acid molecules encoding missense and nonsense mutations, which includes mRNAs, DNAs (e.g., cDNA, genomic DNA, etc.), as well as antisense analogs thereof and diagnostically or therapeutically useful fragments thereof.

This invention also provides recombinant vectors, such as cloning and expression plasmids useful as reagents in the recombinant production of human

galactokinase proteins, as well as recombinant prokaryotic and/or eukaryotic host cells comprising a human galactokinase nucleic acid sequence.

This invention also provides a process for preparing human galactokinase proteins which comprises culturing recombinant prokaryotic and/or eukaryotic host cells, containing a human galactokinase nucleic acid sequence, under conditions promoting expression of said protein and subsequent recovery thereof of said protein. Another related aspect of this invention is isolated human galactokinase proteins produced by said method. In yet another aspect, this invention also provides antibodies that are directed to (i.e., bind) human galactokinase proteins.

This invention also provides an isolated human galactokinase proteins having a missense or nonsense mutation and antibodies (monoclonal or polyclonal) that are specifically reactive with said proteins.

This invention also provides nucleic acid probes and PCR primers comprising nucleic acid molecules of sufficient length to specifically hybridize to human galactokinase sequences.

This invention also provides a method to diagnose human galactokinase deficiency which comprises isolating a nucleic acid sample from an individual and assaying the sequence of said nucleic acid sample with the reference gene of the invention and comparing differences between said sample and the nucleic acid of the instant invention, wherein said differences indicate mutations in the human galactokinase gene isolated from an individual. The sample can be assayed by direct sequence comparison (i.e., DNA sequencing), wherein the sample nucleic acid can be compared to the reference galactokinase gene, by hybridization (e.g., mobility shift assays such as heteroduplex gel electrophoresis, SSCP or other techniques such as Northern or Southern blotting which are based upon the length of the nucleic acid sequence) or other known gel electrophoresis methods such as RLFP (for example, by restriction endonuclease digestion of a sample amplified by PCR (for DNA) or PCR-RT (for RNA)). Alternatively, the diagnostic method comprises isolating cells from an individual containing genomic DNA and assaying said sample (e.g., cellular RNA) by *in situ* hybridization using the DNA sequence of the invention, or at least one exon, or a fragment containing at least 15, preferably 18, and more preferably 21 contiguous base pairs as a probe. This invention also provides an antisense oligonucleotide having a sequence capable of binding with mRNAs encoding human galactokinase so as to identify mutant galactokinase genes.

This invention also provides yet another method to diagnose human galactokinase deficiency which comprises obtaining a serum or tissue sample; allowing such sample to come in contact with an antibody or antibody fragment

which specifically binds to a mutant human galactokinase protein of the invention under conditions such that an antigen-antibody complex is formed between said antibody (or antibody fragment) and said mutant galactokinase protein; and detecting the presence or absence of said complex.

This invention also provides transgenic non-human animals comprising a nucleic acid molecule encoding human galactokinase. Also provided are methods for use of said transgenic animals as models for disease states, mutation and SAR.

This invention also provides a method for treating conditions which are related to insufficient human galactokinase activity which comprises administering to a patient in need thereof a pharmaceutical composition containing the galactokinase protein of the invention which is effective to supplement a patient's endogenous galactokinase and thereby alleviating said condition.

This invention also provides a method for treating conditions which are related to insufficient human galactokinase activity via gene therapy. An additional, or reference, gene comprising the non-mutant galactokinase gene of the instant invention is inserted into a patient's cells either *in vivo* or *ex vivo*. The reference gene is expressed in transfected cells and as a result, the protein encoded by the reference gene corrects the defect (i.e., galactokinase deficiency) thus permitting the transfected cells to function normally and alleviating disease conditions (or symptoms).

Brief Description of the Drawings:

Figure 1 depicts the intron/exon organization of the human galactokinase gene.

Figure 2 is the genomic DNA sequence (and single letter amino acid abbreviations) for human galactokinase [SEQ ID NO: 7]. The bolded DNA sequence corresponds to the exon regions whereas the normal or unbolded type corresponds to the intron regions of human galactokinase.

Detailed Description of the Invention:

This invention relates to human galactokinase (amino acid and nucleotide sequences) and its use as a diagnostic and therapeutic. The particular cDNA and amino acid sequence of human galactokinase is identified by SEQ ID NO:4 as described more fully below. This invention also relates to the genomic DNA sequence for human galactokinase [SEQ ID NO: 7] and also to mutant human galactokinase genes and amino acid sequences [SEQ ID NO:5 and 6] and their use for diagnostic purposes.

In further describing the present invention, the following additional terms will be employed, and are intended to be defined as indicated below.

An "antigen" refers to a molecule containing one or more epitopes that will stimulate a host's immune system to make a humoral and/or cellular antigen-specific response. The term is also used herein interchangeably with "immunogen."

The term "epitope" refers to the site on an antigen or hapten to which a specific antibody molecule binds. The term is also used herein interchangeably with "antigenic determinant" or "antigenic determinant site."

A coding sequence is "operably linked to" another coding sequence when RNA polymerase will transcribe the two coding sequences into a single mRNA, which is then translated into a single polypeptide having amino acids derived from both coding sequences. The coding sequences need not be contiguous to one another so long as the expressed sequence is ultimately processed to produce the desired protein.

"Recombinant" polypeptides refer to polypeptides produced by recombinant DNA techniques; i.e., produced from cells transformed by an exogenous DNA construct encoding the desired polypeptide. "Synthetic" polypeptides are those prepared by chemical synthesis.

A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication in vivo; i.e., capable of replication under its own control.

A "vector" is a replicon, such as a plasmid, phage, or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

A "replication-deficient virus" is a virus in which the excision and/or replication functions have been altered such that after transfection into a host cell, the virus is not able to reproduce and/or infect addition cells.

A "reference" gene refers to the galactokinase sequence of the invention and is understood to include the various sequence polymorphisms that exist, wherein nucleotide substitutions in the gene sequence exist, but do not affect the essential function of the gene product.

A "mutant" gene refers to galactokinase sequences different from the reference gene wherein nucleotide substitutions and/or deletions and/or insertions result in impairment of the essential function of the gene product such that the levels of galactose in an individual (or patient) are atypically elevated. For example, the G to A substitution at position 122 of human galactokinase [SEQ ID NO: 5] is a

missense mutation associated with patients who are galactokinase deficient. Another T for G substitution produces an in-frame nonsense codon at amino acid position 80 of the mature protein. The result is a truncated protein consisting of the first 79 amino acids of human galactokinase.

A DNA "coding sequence of" or a "nucleotide sequence encoding" a particular protein, is a DNA sequence which is transcribed and translated into a polypeptide when placed under the control of appropriate regulatory sequences.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bound at the 3' terminus by a translation start codon (e.g., ATG) of a coding sequence and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

DNA "control sequences" refers collectively to promoter sequences, ribosome binding sites, polyadenylation signals, transcription termination sequences, upstream regulatory domains, enhancers, and the like, which collectively provide for the expression (i.e., the transcription and translation) of a coding sequence in a host cell.

A control sequence "directs the expression" of a coding sequence in a cell when RNA polymerase will bind the promoter sequence and transcribe the coding sequence into mRNA, which is then translated into the polypeptide encoded by the coding sequence.

A "host cell" is a cell which has been transformed or transfected, or is capable of transformation or transfection by an exogenous DNA sequence.

A cell has been "transformed" by exogenous DNA when such exogenous DNA has been introduced inside the cell membrane. Exogenous DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In prokaryotes and yeasts, for example, the exogenous DNA may be maintained on an episomal element, such as a plasmid. With respect to eukaryotic cells, a stably transformed or transfected cell is one in which the exogenous DNA has become integrated into the chromosome so that it is inherited

by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cell containing the exogenous DNA.

"Transfection" or "transfected" refers to a process by which cells take up foreign DNA and integrate that foreign DNA into their chromosome. Transfection can be accomplished, for example, by various techniques in which cells take up DNA (e.g., calcium phosphate precipitation, electroporation, assimilation of liposomes, etc.), or by infection, in which viruses are used to transfer DNA into cells.

A "target cell" is a cell(s) that is selectively transfected over other cell types (or cell lines).

A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth in vitro for many generations.

A "heterologous" region of a DNA construct is an identifiable segment of DNA within or attached to another DNA molecule that is not found in association with the other molecule in nature. Thus, when the heterologous region encodes a gene, the gene will usually be flanked by DNA that does not flank the gene in the genome of the source animal. Another example of a heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., synthetic sequences having codons different from the native gene). Allelic variation or naturally occurring mutational events do not give rise to a heterologous region of DNA, as used herein.

"Conditions which are related to insufficient human galactokinase activity" or a "deficiency in galactokinase activity" means mutations of the galactokinase protein which affects galactokinase activity or may affect expression of galactokinase or both such that the levels of galactose in a patient are atypically elevated. In addition, this definition is intended to cover atypically low levels of galactokinase expression in a patient due to defective control sequences for the reference galactokinase protein.

This invention provides an isolated nucleic acid molecule encoding a human galactokinase protein and substantially similar sequences. Isolated nucleic acid sequences are "substantially similar" if: (i) they are approximately the same length (i.e., at least 80% of the coding region of SEQ ID NO:4); (ii) they encode a protein with the same (i.e., within an order of magnitude) galactokinase activity as the protein encoded by SEQ ID NO:4; and (iii) they are capable of hybridizing under moderately stringent conditions to SEQ ID NO:4; or they encode DNA sequences

which are degenerate to SEQ ID NO:4. Degenerate DNA sequences encode the same amino acid sequence as SEQ ID NO:4, but have variation(s) in the nucleotide coding sequences. Hybridization under moderately stringent conditions is outlined below.

Hybridization under moderately stringent conditions can be performed as follows. Nitrocellulose filters are prehybridized at 65°C in a solution containing 6X SSPE, 5X Denhardt's solution (10g Ficoll, 10g BSA and 10g Polyvinylpyrrolidone per liter solution), 0.05% SDS and 100 micrograms tRNA. Hybridization probes are labeled, preferably radiolabelled (e.g., using the Bios TAG-IT® kit). Hybridization is then carried out for approximately 18 hours at 65°C. The filters are then washed in a solution of 2X SSC and 0.5% SDS at room temperature for 15 minutes (repeated once). Subsequently, the filters are washed at 58°C, air-dried and exposed to X-ray film overnight at -70°C with an intensifying screen.

Alternatively, "substantially similar" sequences are substantially the same when about 66% (preferably about 75%, and most preferably about 90%) of the nucleotides or amino acids match over a defined length (i.e., at least 80% of the coding region of SEQ ID NO:4) of the molecule and the protein encoded by such sequence has the same (i.e., within an order of magnitude) galactokinase activity as the protein encoded by SEQ ID NO:4. As used herein, substantially similar refers to the sequences having similar identity to the sequences of the instant invention. Thus nucleotide sequences that are substantially the same can be identified by hybridization or by sequence comparison. Protein sequences that are substantially the same can be identified by one or more of the following: proteolytic digestion, gel electrophoresis and/or microsequencing.

This invention also provides isolated nucleic acid molecules encoding a missense mutation (SEQ ID NO:5) or a nonsense mutation (SEQ ID NO:6) of the human galactokinase protein and DNA sequences which are degenerate to SEQ ID NO:5 or 6. Degenerate DNA sequences encode the same amino acid (or termination site) sequence as SEQ ID NO:5 or 6, but have variation(s) in the nucleotide coding sequences.

One means for isolating a nucleic acid molecule encoding for a human galactokinase is to probe a human genomic or cDNA library with a natural or artificially designed probe using art recognized procedures (See for example: "Current Protocols in Molecular Biology", Ausubel, F.M., et al. (eds.) Greene Publishing Assoc. and John Wiley Interscience, New York, 1989,1992). It is appreciated to one skilled in the art that SEQ ID NO:4, or fragments thereof (comprising at least 15 contiguous nucleotides), is a particularly useful probe.

Several particularly useful probes for this purpose are set forth in Table 1, or hybridizable fragments thereof (i.e., comprising at least 15 contiguous nucleotides). It is also appreciated that such probes can be and are preferably labeled with an analytically detectable reagent to facilitate identification of the probe. Useful reagents include but are not limited to radioactivity, fluorescent dyes or enzymes capable of catalyzing the formation of a detectable product. The probes are thus useful to isolate complementary copies of genomic DNA, cDNA or RNA from human, mammalian or other animal sources or to screen such sources for related sequences (e.g., additional members of the family, type and/or subtype) and including transcriptional regulatory and control elements defined above as well as other stability, processing, translation and tissue specificity-determining regions from 5' and/or 3' regions relative to the coding sequences disclosed herein.

This invention also provides for gene therapy. "Gene therapy" means gene supplementation. That is, an additional (i.e., reference) copy of the gene of interest is inserted into a patients' cells. As a result, the protein encoded by the reference gene corrects the defect (i.e., galactokinase deficiency) and permits the cells to function normally thus alleviating disease symptoms.

Gene therapy of the present invention can occur *in vivo* or *ex vivo*. *Ex vivo* gene therapy requires the isolation and purification of patient cells, the introduction of a therapeutic gene, and introduction of the genetically altered cells back into the patient. A replication-deficient virus such as a modified retrovirus can be used to introduce the therapeutic gene (galactokinase) into such cells. For example, mouse Moloney leukemia virus (MMLV) is a well-known vector in clinical gene therapy trials (see, e.g., Boris-Lauerie et al., Curr. Opin. Genet. Dev., 3:102-109 (1993)).

In contrast, *in vivo* gene therapy does not require isolation and purification of patients' cells. The therapeutic gene is typically "packaged" for administration to a patient such as in liposomes or in a replication-deficient virus such as adenovirus (see, e.g., Berkner, K.L., Curr. Top. Microbiol. Immunol., 158:39-66 (1992)) or adeno-associated virus (AAV) vectors (see, e.g., Muzyczka, N., Curr. Top. Microbiol. Immunol., 158:97-129 (1992) and U.S. Patent 5,252,479 "Safe Vector for Gene Therapy"). Another approach is administration of so-called "naked DNA" in which the therapeutic gene is directly injected into the bloodstream or muscle tissue.

Cell types useful for gene therapy of the present invention include hepatocytes, fibroblasts, lymphocytes, any cell of the eye (e.g., retina), epithelial and endothelial cells. Preferably the cells are hepatocytes, any cell of the eye or respiratory (or pulmonary) epithelial cells. Transfection of (pulmonary) epithelial

cells can occur via inhalation of a neubulized preparation of DNA vectors in liposomes, DNA-protein complexes or replication-deficient adenoviruses (see, e.g., U.S. Patent 5,240,846 "Gene Therapy Vector for Cystic Fibrosis".

This invention also provides for a process to prepare human galactokinase proteins. Non-mutant proteins are defined with reference to the amino acid sequence listed in SEQ ID NO:4 and includes variants with a substantially similar amino acid sequence that have the same galactokinase activity. Additional proteins of this invention include mutant human galactokinase proteins as set forth in SEQ ID NO: 5 or 6. The proteins of this invention are preferably made by recombinant genetic engineering techniques. The isolated nucleic acids particularly the DNAs can be introduced into expression vectors by operatively linking the DNA to the necessary expression control regions (e.g., regulatory regions) required for gene expression. The vectors can be introduced into the appropriate host cells such as prokaryotic (e.g., bacterial), or eukaryotic (e.g., yeast or mammalian) cells by methods well known in the art (Ausubel et al., supra). The coding sequences for the desired proteins having been prepared or isolated, can be cloned into any suitable vector or replicon. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. Examples of recombinant DNA vectors for cloning and host cells which they can transform include, but is not limited to, the bacteriophage λ (E. coli), pBR322 (E. coli), pACYC177 (E. coli), pKT230 (gram-negative bacteria), pGV1106 (gram-negative bacteria), pLAFR1 (gram-negative bacteria), pME290 (non-E. coli gram-negative bacteria), pHV14 (E. coli and Bacillus subtilis), pBD9 (Bacillus), pIJ61 (Streptomyces), pUC6 (Streptomyces), YIp5 (Saccharomyces), a baculovirus insect cell system, a Drosophila insect system, and YCp19 (Saccharomyces). See, generally, "DNA Cloning": Vols. I & II, Glover et al. ed. IRL Press Oxford (1985) (1987) and; T. Maniatis et al. ("Molecular Cloning" Cold Spring Harbor Laboratory (1982).

The gene can be placed under the control of a promoter, ribosome binding site (for bacterial expression) and, optionally, an operator (collectively referred to herein as "control" elements), so that the DNA sequence encoding the desired protein is transcribed into RNA in the host cell transformed by a vector containing this expression construction. The coding sequence may or may not contain a signal peptide or leader sequence. The subunit antigens of the present invention can be expressed using, for example, the E. coli tac promoter or the protein A gene (spa) promoter and signal sequence. Leader sequences can be removed by the bacterial host in post-translational processing. See, e.g., U.S. Patent Nos. 4,431,739; 4,425,437; 4,338,397.

In addition to control sequences, it may be desirable to add regulatory sequences which allow for regulation of the expression of the protein sequences relative to the growth of the host cell. Regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector, for example, enhancer sequences.

An expression vector is constructed so that the particular coding sequence is located in the vector with the appropriate regulatory sequences, the positioning and orientation of the coding sequence with respect to the control sequences being such that the coding sequence is transcribed under the "control" of the control sequences (i.e., RNA polymerase which binds to the DNA molecule at the control sequences transcribes the coding sequence). Modification of the sequences encoding the particular antigen of interest may be desirable to achieve this end. For example, in some cases it may be necessary to modify the sequence so that it may be attached to the control sequences with the appropriate orientation; i.e., to maintain the reading frame. The control sequences and other regulatory sequences may be ligated to the coding sequence prior to insertion into a vector, such as the cloning vectors described above. Alternatively, the coding sequence can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site.

In some cases, it may be desirable to produce other mutants or analogs of the galactokinase protein. Mutants or analogs may be prepared by the deletion of a portion of the sequence encoding the protein, by insertion of a sequence, and/or by substitution of one or more nucleotides within the sequence. Techniques for modifying nucleotide sequences, such as site-directed mutagenesis, are well known to those skilled in the art. See, e.g., T. Maniatis et al., supra; DNA Cloning, Vols. I and II, supra; Nucleic Acid Hybridization, supra.

A number of prokaryotic expression vectors are known in the art. See, e.g., U.S. Patent Nos. 4,578,355; 4,440,859; 4,436,815; 4,431,740; 4,431,739; 4,428,941; 4,425,437; 4,418,149; 4,411,994; 4,366,246; 4,342,832; see also U.K. Patent Applications GB 2,121,054; GB 2,008,123; GB 2,007,675; and European Patent Application 103,395. Yeast expression vectors are also known in the art. See, e.g., U.S. Patent Nos. 4,446,235; 4,443,539; 4,430,428; see also European Patent Applications 103,409; 100,561; 96,491. pSV2neo (as described in J. Mol. Appl. Genet. 1:327-341) which uses the SV40 late promoter to drive expression in mammalian cells or pCDNA1neo, a vector derived from pCDNA1 (Mol. Cell Biol.

7:4125-29) which uses the CMV promoter to drive expression. Both these latter two vectors can be employed for transient or stable (using G418 resistance) expression in mammalian cells. Insect cell expression systems, e.g., Drosophila, are also useful, see for example, PCT applications WO 90/06358 and WO 92/06212 as well as EP 290,261-B1.

Depending on the expression system and host selected, the proteins of the present invention are produced by growing host cells transformed by an expression vector described above under conditions whereby the protein of interest is expressed. Preferred mammalian cells include human embryonic kidney cells, monkey kidney (HEK-293cells), fibroblast (COS) cells, Chinese hamster ovary (CHO) cells, Drosophila or murine L-cells. If the expression system secretes the protein into growth media, the protein can be purified directly from the media. If the protein is not secreted, it is isolated from cell lysates or recovered from the cell membrane fraction. The selection of the appropriate growth conditions and recovery methods are within the skill of the art.

An alternative method to identify proteins of the present invention is by constructing gene libraries, using the resulting clones to transform E. coli and pooling and screening individual colonies using polyclonal serum or monoclonal antibodies to galactokinase.

The proteins of the present invention may also be produced by chemical synthesis such as solid phase peptide synthesis, using known amino acid sequences or amino acid sequences derived from the DNA sequence of the genes of interest. Such methods are known to those skilled in the art. Chemical synthesis of peptides is not particularly preferred.

The proteins of the present invention or their fragments comprising at least one epitope can be used to produce antibodies, both polyclonal and monoclonal. If polyclonal antibodies are desired, a selected mammal, (e.g., mouse, rabbit, goat, horse, etc.) is immunized with the protein of the present invention, or a fragment thereof, capable of eliciting an immune response (i.e., having at least one epitope). Serum from the immunized animal is collected and treated according to known procedures. If serum containing polyclonal antibodies is used, the polyclonal antibodies can be purified by immunoaffinity chromatography or other known procedures.

Monoclonal antibodies to the proteins of the present invention, and to the fragments thereof, can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies by using hybridoma technology is well known. Immortal antibody-producing cell lines can be created by

cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. See, e.g., M. Schreier et al., "Hybridoma Techniques" (1980); Hammerling et al., "Monoclonal Antibodies and T-cell Hybridomas" (1981); Kennett et al., "Monoclonal Antibodies" (1980); see also U.S. Patent Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,452,570; 4,466,917; 4,472,500; 4,491,632; and 4,493,890. Panels of monoclonal antibodies produced against the antigen of interest, or fragment thereof, can be screened for various properties; i.e., for isotype, epitope, affinity, etc. Hence one skilled in the art can produce monoclonal antibodies specifically reactive with mutant galactokinase proteins, e.g., the missense mutation of SEQ ID NO:5 or nonsense mutation of SEQ ID NO:6. Monoclonal antibodies are useful in purification, using immunoaffinity techniques, of the individual antigens which they are directed against. Alternatively, genes encoding the monoclonals of interest may be isolated from the hybridomas by PCR techniques known in the art and cloned and expressed in the appropriate vectors. The antibodies of this invention, whether polyclonal or monoclonal have additional utility in that they may be employed reagents in immunoassays, RIA, ELISA, and the like. As used herein, "monoclonal antibody" is understood to include antibodies derived from one species (e.g., murine, rabbit, goat, rat, human, etc.) as well as antibodies derived from two (or perhaps more) species (e.g., chimeric and humanized antibodies).

Chimeric antibodies, in which non-human variable regions are joined or fused to human constant regions (see, e.g. Liu et al., Proc. Natl Acad. Sci. USA, 84:3439 (1987)), may also be used in assays or therapeutically. Preferably, a therapeutic monoclonal antibody would be "humanized" as described in Jones et al., Nature, 321:522 (1986); Verhoeyen et al., Science, 239:1534 (1988); Kabat et al., J. Immunol., 147:1709 (1991); Queen et al., Proc. Natl Acad. Sci. USA, 86:10029 (1989); Gorman et al., Proc. Natl Acad. Sci. USA, 88:34181 (1991); and Hodgson et al., Bio/Technology, 9:421 (1991). Therefore, this invention also contemplates antibodies, polyclonal or monoclonal (including chimeric and "humanized") directed to epitopes corresponding to amino acid sequences disclosed herein from human galactokinase. Methods for the production of polyclonal and monoclonal antibodies are well known, see for example Chap. 11 of Ausubel et al. (supra).

When the antibody is labeled with an analytically detectable reagent such a radioactivity, fluorescence, or an enzyme, the antibody can be use to detect the presence or absence of human galactokinase and/or its quantitative level. In addition, antibodies (polyclonal or monoclonal) specific for the missense and nonsense mutations of the present invention are useful for diagnostic purposes. A serum or

tissue sample (e.g., liver, lung, etc.) is obtained and allowed to come in contact with an antibody or antibody fragment which specifically binds to a mutant human galactokinase protein of the invention under conditions such that an antigen-antibody complex is formed between said antibody (or antibody fragment) and said mutant galactokinase protein. The detection for the presence or absence of said complex is within the skill of the art (e.g., ELISA, RIA, Western Blotting, Optical Biosensor (e.g., BIAcore - Pharmacia Biosensor, Uppsala, Sweden) and do not limit this invention.

This invention also contemplates pharmaceutical compositions comprising an effective amount of the galactokinase protein of the invention and a pharmaceutically acceptable carrier. Pharmaceutical compositions of proteinaceous drugs of this invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly or intravenously. Optionally, the galactokinase protein is surrounded by a membrane bound vesicle, such as a liposome.

The compositions for parenteral administration will commonly comprise a solution of the compounds of the invention or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be employed, e.g., water, buffered water, 0.4% saline, 0.3% glycine, and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the compound of the invention in such pharmaceutical formulation can very widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected.

Thus, a pharmaceutical composition of the invention for intramuscular injection could be prepared to contain 1 mL sterile buffered water, and 50 mg of a compound of the invention. Similarly, a pharmaceutical composition of the invention for intravenous infusion could be made up to contain 250 ml of sterile Ringer's solution, and 150 mg of a compound of the invention. Actual methods for preparing parenterally administrable compositions are well known or will be apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania.

The compounds described herein can be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be

effective with conventional proteins and art-known lyophilization and reconstitution techniques can be employed.

The physician will determine the dosage of the present therapeutic agents which will be most suitable and it will vary with the form of administration and the particular compound chosen, and furthermore, it will vary with the particular patient under patient under treatment. He will generally wish to initiate treatment with small dosages substantially less than the optimum dose of the compound and increase the dosage by small increments until the optimum effect under the circumstances is reached. It will generally be found that when the composition is administered orally, larger quantities of the active agent will be required to produce the same effect as a smaller quantity given parenterally. The therapeutic dosage will generally be from 1 to 10 milligrams per day and higher although it may be administered in several different dosage units.

Depending on the patient condition, the pharmaceutical composition of the invention can be administered for prophylactic and/or therapeutic treatments. In therapeutic application, compositions are administered to a patient already suffering from a disease in an amount sufficient to cure or at least partially arrest the disease and its complications. In prophylactic applications, compositions containing the present compounds or a cocktail thereof are administered to a patient not already in a disease state to enhance the patient's resistance.

Single or multiple administrations of the pharmaceutical compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical composition of the invention should provide a quantity of the compounds of the invention sufficient to effectively treat the patient.

This invention also contemplates use of the galactokinase genes of the instant invention as a diagnostic. For example, some diseases result from inherited defective genes. These genes can be detected by comparing the sequence of the defective gene with that of a normal one. Subsequently, one can verify that a "mutant" gene is associated with galactokinase deficiency by measurement of galactose. That is, a mutant gene would be associated with (atypically) elevated levels of galactose in a patient. In addition, one can insert mutant galactokinase genes into a suitable vector for expression in a functional assay system (e.g., colorimetric assay, expression on MacConkey plates, complementation experiments, e.g, in a galactokinase deficient strain of yeast or *E. coli*) as yet another means to verify or identify galactokinase mutations. As an example, RNA from an individual can be transcribed with reverse transcriptase to cDNA which can then be amplified by polymerase chain reaction (PCR), cloned into an *E. coli* expression vector, and

transformed into a galactokinase-deficient strain of *E. coli*. When grown on MacConkey indicator plates, galactokinase-deficient cells will produce colonies that are white in color, whereas cells that have been transformed/complemented with a functional galactokinase gene will be red (see, e.g., Examples section). If most to all of the colonies from an individual are red, then the individual is considered to be normal with respect to galactokinase activity. If approximately 50% of the colonies are red (the other 50% white), then that individual is likely to be a carrier for galactokinase deficiency. If most to all of the colonies are white, then that individual is likely to be galactokinase deficient. Once "mutant" genes have been identified, one can then screen the population for carriers of the "mutant" galactokinase gene. (A carrier is a person in apparent health whose chromosomes contain a "mutant" galactokinase gene that may be transmitted to that person's offspring.) In addition, monoclonal antibodies that are specific for the mutant galactokinase proteins can be used for diagnostic purposes as described above.

Individuals carrying mutations in the human galactokinase gene may be detected at the DNA level by a variety of techniques. Nucleic acids used for diagnosis (genomic DNA, mRNA, etc.) may be obtained from a patient's cells, such as from blood, urine, saliva, tissue biopsy (e.g., chorionic villi sampling or removal of amniotic fluid cells), and autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR, ligase chain reaction (LCR), strand displacement amplification (SDA), etc. (see, e.g., Saiki et al., Nature, 324:163-166 (1986), Bej, et al., Crit. Rev. Biochem. Molec. Biol., 26:301-334 (1991), Birkenmeyer et al., J. Virol. Meth., 35:117-126 (1991), Van Brunt, J., Bio/Technology, 8:291-294 (1990)) prior to analysis. RNA may also be used for the same purpose. The RNA can be reverse-transcribed and amplified at one time with PCR-RT (polymerase chain reaction - reverse transcriptase) or reverse-transcribed to an unamplified cDNA. As an example, PCR primers complementary to the nucleic acid of the instant invention can be used to identify and analyze galactokinase mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal galactokinase genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled galactokinase RNA (of the invention) or alternatively, radiolabelled galactokinase antisense DNA sequences (of the invention). Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures (Tm). Such a diagnostic would be particularly useful for prenatal and even neonatal testing.

In addition, point mutations and other sequence differences between the reference gene and "mutant" genes can be identified by yet other well-known techniques, e.g., direct DNA sequencing, single-strand conformational polymorphism (SSCP; Orita et al., Genomics, 5:874-879 (1989)). For example, a sequencing primer is used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabeled nucleotides or by automatic sequencing procedures with fluorescent-tags. Cloned DNA segments may also be used as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. The presence of nucleotide repeats may correlate to a change in galactokinase activity (causative change) or serve as marker for various polymorphisms.

Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, e.g., Myers et al., Science, 230:1242 (1985)). In addition, sequence alterations, in particular small deletions, may be detected as changes in the migration pattern of DNA heteroduplexes in non-denaturing gel electrophoresis (i.e., heteroduplex electrophoresis) (see, e.g., Nagamine et al., Am. J. Hum. Genet., 45:337-339 (1989)).

Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (e.g., Cotton et al., Proc. Natl. Acad. Sci. USA, 85:4397-4401 (1985)).

Thus, the detection of a specific DNA sequence may be achieved by methods such as hybridization (e.g., heteroduplex electroporation, see, White et al., Genomics, 12:301-306 (1992), RNAse protection (e.g., Myers et al., Science, 230:1242 (1985)) chemical cleavage (e.g., Cotton et al., Proc. Natl. Acad. Sci. USA, 85:4397-4401 (1985))), direct DNA sequencing, or the use of restriction enzymes (e.g., restriction fragment length polymorphisms (RFLP) in which variations in the number and size of restriction fragments can indicate insertions, deletions, presence of nucleotide repeats and any other mutation which creates or destroys an endonuclease restriction sequence). Southern blotting of genomic DNA may also be used to identify large (i.e., greater than 100 base pair) deletions and insertions.

In addition to more conventional gel-electrophoresis, and DNA sequencing, mutations (e.g., microdeletions, aneuploidies, translocations, inversions) can also be detected by *in situ* analysis (See, e.g., Keller et al., DNA Probes, 2nd Ed., Stockton Press, New York, N.Y., USA (1993)). That is, DNA (or RNA) sequences in cells can be analyzed for mutations without isolation and/or immobilization onto a membrane. Fluorescence *in situ* hybridization (FISH) is presently the most commonly applied method and numerous reviews of FISH have appeared. See, e.g., Trachuck et al., Science, 250:559-562 (1990), and Trask et al., Trends. Genet., 7: 149-154 (1991) which are incorporated herein by reference for background purposes. Hence, by using nucleic acids based on the structure of specific genes, e.g., galactokinase, one can develop diagnostic tests for galactokinase deficiency.

In addition, some diseases are a result of, or are characterized by, changes in gene expression which can be detected by changes in the mRNA. Alternatively, the galactokinase gene can be used as a reference to identify individuals expressing a decreased level of galactokinase, e.g., by Northern blotting or *in situ* hybridization.

Defining appropriate hybridization conditions is within the skill of the art. See, e.g., "Current Protocols in Mol. Biol." Vol. I & II, Wiley Interscience. Ausbel et al. (ed.) (1992). Probing technology is well known in the art and it is appreciated that the size of the probes can vary widely but it is preferred that the probe be at least 15 nucleotides in length. It is also appreciated that such probes can be and are preferably labeled with an analytically detectable reagent to facilitate identification of the probe. Useful reagents include but are not limited to radioactivity, fluorescent dyes or enzymes capable of catalyzing the formation of a detectable product. As a general rule the more stringent the hybridization conditions the more closely related genes will be that are recovered.

Also within the scope of this invention are antisense oligonucleotides predicated upon the sequences disclosed herein for human galactokinase. Synthetic oligonucleotides or related antisense chemical structural analogs are designed to recognize and specifically bind to a target nucleic acid encoding galactokinase and galactokinase mutations. The general field of antisense technology is illustrated by the following disclosures which are incorporated herein by reference for purposes of background (Cohen, J.S., Trends in Pharm. Sci., 10:435(1989) and Weintraub, H.M. Scientific American, Jan.(1990) at page 40).

Transgenic, non-human, animals may be obtained by transfecting appropriate fertilized eggs or embryos of a host with nucleic acids encoding human galactokinase disclosed herein, see for example U.S. Patents 4,736,866; 5,175,385; 5,175,384 and 5,175,386. The resultant transgenic animal may be used as a model for the study of

galactokinase. Particularly, useful transgenic animals are those which display a detectable phenotype associated with the expression of the receptor. Drugs may then be screened for their ability to reverse or exacerbate the relevant phenotype. This invention also contemplates operatively linking the receptor coding gene to regulatory elements which are differentially responsive to various temperature or metabolic conditions, thereby effectively turning on or off the phenotypic expression in response to those conditions.

Although not necessarily limiting of this invention, following are some experimental data illustrative of this invention.

EXAMPLE I

Purification of Human Galactokinase from Placental Tissue

Galactokinase (galK) was obtained from human placenta as described by Stambolian et al. (Biochim Biophys Acta, 831:306-312 (1985)), which is incorporated by reference in its entirety. In essence, human placenta tissue (obtained within 1 hour of parturition) was homogenized, centrifuged and the resulting supernatant was absorbed onto DEAE-Sephacel®. The material was eluted, precipitated with ammonium sulfate and then run through a sizing column (Sephadex G-100 SF®). Pooled active fractions were concentrated. Purified protein was obtained following separation by SDS polyacrylamide electrophoresis and then Western blotted using standard techniques (see, Laemmli, Nature, 227:680-685 (1970), or LeGendre et al., Biotechniques, 6:154 (1988)). Minute amounts of galactokinase were isolated (micrograms) from multiple rounds of protein purification. After a trypsin peptide digest, 7 peptide sequences were eventually isolated and identified. The three longest fragments are presented below:

[SEQ ID NO:1]
Val Asn Leu Ile Gly Glu His Thr Asp Tyr Asn Gln Gly Leu Val Leu-
Pro Met Ala Leu Glu Leu Met Thr Val Leu Val Gly Ser Pro Arg

[SEQ ID NO:2]
His Ile Gln Glu His Tyr Gly Gly Thr Ala Thr Phe Tyr Leu Ser Gln-
Ala Ala Asp Gly Ala Lys

[SEQ ID NO:3]
Ala Gln Val Cys Gln Gln Ala Glu His Ser Phe Ala Gly Met Pro Cys-
Gly Ile Met Asp Gln Phe Ile Ser Leu Met Gly Gln Lys

The fragments were compared with peptide sequences encoded by cDNAs, in which the cDNAs were partially sequenced. The cDNAs (also known as expressed sequence tags or ESTs) were obtained from Human Genome Sciences, Inc. (Rockville, MD, USA). The best alignments occurred with an EST sequence from a human osteoclastoma stromal cell library (SEQ ID NO:1 showed 100% identity over 18 contiguous amino acids) and an EST sequence from a human pituitary library (SEQ ID NO:2 showed 95.5% identity over 22 contiguous amino acids). A full-length cDNA from the human osteoclastoma stromal cell library was identified and sequenced (SEQ ID NO:4) in its entirety on an automated ABI 373A Sequencer. Sequencing was confirmed on both strands. The corresponding amino acid sequence (SEQ ID NO:4) was compared against the peptide fragments identified above. SEQ ID NO:1 corresponds to amino acids 38-68 of the full-length human galactokinase protein. Similarly, SEQ ID NOs: 2 and 3 correspond to amino acids 367-388 and 167-195, respectively, of human galactokinase.

Analysis of the Human Galactokinase Gene:

A comparison of the amino acid sequence for human galactokinase with that of *E. coli* galactokinase (Debouck et al., Nuc. Acid Res., 13:1841-1853 (1985)) shows 61% similarity and 44.5% identity. Further comparison with another purported human galactokinase gene (*GK2*) (Lee et al., Proc. Natl. Acad. Sci. USA, 89:10887-10891 (1992)) shows 54% similarity and 34.6% identity at the amino acid level. Furthermore, the *GK2* gene maps to human chromosome 15 which is in contrast to the gene of the present invention which maps to human chromosome 17, position q24 as determined by fluorescence *in situ* hybridization (FISH) analysis.

SEQ ID NO:4 was hybridized against a Northern blot containing human messenger RNA from placenta, brain, skeletal muscle, kidney, intestine, heart, lung and liver according to standard procedures (see, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, 1989). Hybridization was strongest with human liver and lung tissue.

Galactokinase Complementation:

SEQ ID NO:4 was subcloned into an *E. coli* vector, plasmid pBluescript [Stratagene]. When transformed into C600K-, a galactokinase-deficient strain, the transformed *E. coli* grew on MacConkey agar plates containing 1% galactose (and ampicillin @ 50ug/ml for plasmid selection), and produced brick red colonies, indicating sugar fermentation. Specifically, the red color is due to the action of acids,

produced by galactose fermentation, upon bile salts and the indicator (neutral red) in MacConkey medium.

Expression in Mammalian Cells:

SEQ ID NO:4 was also subcloned into COS-1 cells [ATCC CRL 1650]. The cells were transfected, grown, and cell lysates were prepared. The lysates were assayed by a ^{14}C galactokinase assay as described by Stambolian et al. (Exp. Eye Res., 38:231-237 (1984)) which is hereby incorporated by reference in its entirety. When expressed in transiently transfected COS cells, galactokinase activity was tenfold higher than control levels (6600 vs. 640 counts per minute - repeated three times). These results definitively confirm that SEQ ID NO:4 encodes a full-length, biologically active, human galactokinase gene.

The nucleic acid molecule of the invention can also be subcloned into an expression vector to produce high levels of human galactokinase (either fused to another protein, e.g., operatively linked at the 5' end with another coding sequence, or unfused) in transfected cells. For mammalian cells, the expression vector would optionally encode a neomycin resistance gene to select for transfectants on the basis of ability to grow in G418 and a dihydrofolate reductase gene which permits amplification of the transfected gene in $DHFR^-$ cells. The plasmid can then be introduced into host cell lines e.g., CHO ACC98, a nonadherent, $DHFR^-$ cell line adapted to grow in serum free medium, and human embryonic kidney 293 cells (ATCC CRL 1573), and transfected cell lines can be selected by G418 resistance.

Human Galactokinase Gene - Genomic Sequence:

A full-length galactokinase genomic gene coding region was identified from a lambda phage (λ Fix II) human genomic library (made from human placenta tissue) using the galK cDNA as a probe. One isolate, designated clone 17 was deposited on 3 May 1995, with the American Type Culture Collection (ATCC), Rockville, MD, USA, under accession number ATCC 97135, and has been accepted as a patent deposit, in accordance with the Budapest Treaty of 1977 governing the deposit of microorganisms for the purposes of patent procedure.

The genomic gene coding region is divided into at least 8 exons isolated from 4 DNA fragments. The arrangement is depicted in Figure 1. The DNA sequence was determined by using multiple oligonucleotide PCR primers corresponding to the galK cDNA sequence (i.e., corresponding to galK genomic exons) as well as oligonucleotide PCR primers subsequently designed that correspond to non-coding regions (i.e., galK genomic introns). Thus the structure of the galactokinase genomic gene is summarized in Table 1 below (see also Figure 2 and SEQ ID NO:7]):

Table 1
Genomic Galactokinase Gene

Exon #	Amino Acids Encoded	PCR Primer #/ [SEQ ID NO]
1	1-55	3333/[8] 3334/[9] 3598/[10] 3599/[11]
2	56-118	1888/[12] 3332/[13] 3604/[14] 3605/[15]
3	119-158	3331/[16] 3606/[17]
4	159-204	1657/[18] 3034/[19]
5	205-264	3330/[20] 3607/[21]
6	265-315	1539/[22] 2665/[23]
7	316-369	1891/[24] 2665/[25]
8	370-392	2665/[26] 2666/[27] 2667/[28]

Galactokinase Deficiency Marker/Gene:

A fibroblast cell line (GM00334), derived from a patient with galactokinase deficiency, was obtained from the Coriell Institute for Medical research, 401 Haddon

Ave., Camden, New Jersey, 08103. Total RNA was isolated from the cultured cells using the RNAZOL kit for isolation of RNA (Biotecx, Houston, Tx). Cytoplasmic DNA (1 ug) was reversed transcribed with oligonucleotide primers 1823 [SEQ ID NO: 29] and 1825 [SEQ ID NO: 30]. The sample was amplified by 35 cycles at 94°C for 1 min., 60°C for 1 min. and 72°C for 7 min. The DNA product was purified electrophoretically, ligated to the TA cloning vector (Invitrogen) and sequenced. Twelve cDNAs in total were sequenced (representing cloned PCR products of multiple independent PCR reactions). This procedure was also repeated with cultured fibroblasts from normal controls (i.e., persons not exhibiting galactokinase deficiency).

A comparison with normal controls identified a single base substitution of A for G at position 122 of the "normal" human galactokinase gene [SEQ ID NO: 4]. The result is a missense mutation in amino acid 32 from Val to Met [SEQ ID NO: 5]. The G to A base change creates a MscI endonuclease restriction site (i.e., TGG↓CCA) on the mutant allele. This restriction site was then used to rapidly screen for the mutant allele in the parents of the patient with galactokinase deficiency. In essence, the exon encoding galactokinase residues 1 to 5 (i.e., exon 1, see Table 1) was cloned from a genomic lambda phage library and its DNA sequence was determined, including a portion of the flanking intron sequences. Oligonucleotide primers (X2-5OUT [SEQ ID NO: 31] and X2-3OUT [SEQ ID NO: 32]) were designed to hybridize to intron sequences for the amplification of a 346 bp DNA fragment of the genomic DNA. The PCR product was analyzed for the point mutation via RFLP, that is, the presence of a newly created MscI site as detected by electrophoresis of a 1.5% agarose gel. A "normal" allele remains uncut with the enzyme MscI, and thus migrates as a 346bp fragment on an agarose gel. The PCR product from the patient with galactokinase deficiency (i.e., the G to A base change) is cleaved with MscI, resulting in two fragments of 193 and 153 bp, respectively. The absence of 346 bp fragment indicates that the patient was homozygous for this allele. In contrast, PCR products from the parents of this patient, followed by a MscI digestion, resulted in three fragments (346, 193 and 153 bp) which is consistent with a heterozygous pattern for the G to A base change. That is, the parents were both carriers of the same mutation.

To determine whether the missense mutation resulted in decreased enzymatic activity, a cDNA clone containing the G to A base change was subcloned into COS cells and assayed for galactokinase activity as previously described. COS cells transfected with cDNA encoding the missense mutation had the same level of galactokinase activity as the host COS cells, namely 0.02 units/ug protein. In contrast, COS cells transfected with the non-mutant galactokinase cDNA [SEQ ID NO:4] had a

fifty-fold higher activity compared to the host COS cells (i.e., control). This results supports the Val^{32} to Met^{32} substitution as the cause of the decreased enzymatic activity.

Another mutation was discovered in an unrelated patient having cataracts and diagnosed as galactokinase deficient (galactokinase activity was found to be close to zero). Genomic DNA was isolated from lymphoblastoid cell lines and sequenced by automated sequencing on an ABI 373A sequencer. A single base substitution of T for G resulted in an in-frame nonsense codon (i.e., TAG) at amino acid position 80 [SEQ ID NO:6]. This mutation causes premature termination of human galactokinase, resulting in a truncated protein of 79 amino acids that would be expected to be non-functional. (The genomic DNA of the parents of this patient were heterozygous for this mutation, and hence not galactokinase deficient.)

The above description and examples fully disclose the invention including preferred embodiments thereof. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments herein. Such equivalents are intended to be within the scope of the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Bergsma, Derk J.
Stambolian, Dwight

(ii) TITLE OF INVENTION: Human Galactokinase Gene

(iii) NUMBER OF SEQUENCES: 32

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: SmithKline Beecham Corp./Corporate Intellectual Property
(B) STREET: 709 Swedeland Road/UW2220
(C) CITY: King of Prussia
(D) STATE: Pennsylvania
(E) COUNTRY: USA
(F) ZIP: 19406-0939

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US94/10825
(B) FILING DATE: 23-SEP-1994

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Sutton, Jeffrey A.
(B) REGISTRATION NUMBER: 34,028
(C) REFERENCE/DOCKET NUMBER: P50268-1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 610-270-5024
(B) TELEFAX: 610-270-5090

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Val Asn Leu Ile Gly Glu His Thr Asp Tyr Asn Gln Gly Leu Val Leu
1 5 10 15

Pro Met Ala Leu Glu Leu Met Thr Val Leu Val Gly Ser Pro Arg
20 25 30

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

His Ile Gln Glu His Tyr Gly Gly Thr Ala Thr Phe Tyr Leu Ser Gln
1 5 10 15

Ala Ala Asp Gly Ala Lys
20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ala Gln Val Cys Gln Gln Ala Glu His Ser Phe Ala Gly Met Pro Cys
1 5 10 15

Gly Ile Met Asp Gln Phe Ile Ser Leu Met Gly Gln Lys
20 25

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1349 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 29..1204

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GAATTCGGCA CGAGTGCAGG CGCGCGTC ATG GCT GCT TTG AGA CAG CCC CAG
52
Met Ala Ala Leu Arg Gln Pro Gln
1 5

GTC GCG GAG CTG CTG GCC GAG GCC CGG CGA GCC TTC CGG GAG GAG TTC
100
Val Ala Glu Leu Leu Ala Glu Ala Arg Arg Ala Phe Arg Glu Glu Phe
10 15 20

GGG GCC GAG CCC GAG CTG GCC GTG TCA GCG CCG GGC CGC GTC AAC CTC
148
Gly Ala Glu Pro Glu Leu Ala Val Ser Ala Pro Gly Arg Val Asn Leu
25 30 35 40

ATC GGG GAA CAC ACG GAC TAC AAC CAG GGC CTG GTG CTG CCT ATG GCT
196
Ile Gly Glu His Thr Asp Tyr Asn Gln Gly Leu Val Leu Pro Met Ala
45 50 55

CTG GAG CTC ATG ACG GTG CTG GTG GGC AGC CCC CGC AAG GAT GGG CTG
244
Leu Glu Leu Met Thr Val Leu Val Gly Ser Pro Arg Lys Asp Gly Leu
60 65 70

GTG TCT CTC CTC ACC ACC TCT GAG GGT GCC GAT GAG CCC CAG CGG CTG
292
Val Ser Leu Leu Thr Thr Ser Glu Gly Ala Asp Glu Pro Gln Arg Leu
75 80 85

CAG TTT CCA CTG CCC ACA GCC CAG CGC TCG CTG GAG CCT GGG ACT CCT
340
Gln Phe Pro Leu Pro Thr Ala Gln Arg Ser Leu Glu Pro Gly Thr Pro

90 95 100

CGG TGG GCC AAC TAT GTC AAG GGA GTG ATT CAG TAC TAC CCA GCT GCC
388
Arg Trp Ala Asn Tyr Val Lys Gly Val Ile Gln Tyr Tyr Pro Ala Ala
105 110 115 120

CCC CTC CCT GGC TTC AGT GCA GTG GTG GTC AGC TCA GTG CCC CTG GGG
436
Pro Leu Pro Gly Phe Ser Ala Val Val Val Ser Ser Val Pro Leu Gly
125 130 135

GGT GGC CTG TCC AGC TCA GCA TCC TTG GAA GTG GCC ACG TAC ACC TTC
484
Gly Gly Leu Ser Ser Ser Ala Ser Leu Glu Val Ala Thr Tyr Thr Phe
140 145 150

CTC CAG CAG CTC TGT CCA GAC TCG GGC ACA ATA GCT GCC CGC GCC CAG
532
Leu Gln Gln Leu Cys Pro Asp Ser Gly Thr Ile Ala Ala Arg Ala Gln
155 160 165

GTG TGT CAG CAG GCC GAG CAC AGC TTC GCA GGG ATG CCC TGT GGC ATC
580
Val Cys Gln Gln Ala Glu His Ser Phe Ala Gly Met Pro Cys Gly Ile
170 175 180

ATG GAC CAG TTC ATC TCA CTT ATG GGA CAG AAA GGC CAC GCG CTG CTC
628
Met Asp Gln Phe Ile Ser Leu Met Gly Gln Lys Gly His Ala Leu Leu
185 190 195 200

ATT GAC TGC AGG TCC TTG GAG ACC AGC CTG GTG CCA CTC TCG GAC CCC
676
Ile Asp Cys Arg Ser Leu Glu Thr Ser Leu Val Pro Leu Ser Asp Pro
205 210 215

AAG CTG GCC GTG CTC ATC ACC AAC TCT AAT GTC CGC CAC TCC CTG GCC
724
Lys Leu Ala Val Leu Ile Thr Asn Ser Asn Val Arg His Ser Leu Ala

220 225 230

TCC AGC GAG TAC CCT GTG CGG CGG CGC CAA TGT GAA GAA GTG GCC CGG
772
Ser Ser Glu Tyr Pro Val Arg Arg Arg Gln Cys Glu Glu Val Ala Arg
235 240 245

GCG CTG GGC AAG GAA AGC CTC CGG GAG GTA CAA CTG GAA GAG CTA GAG
820
Ala Leu Gly Lys Glu Ser Leu Arg Glu Val Gln Leu Glu Glu Leu Glu
250 255 260

GCT GCC AGG GAC CTG GTG AGC AAA GAG GGC TTC CGG CGG GCC CGG CAC
868
Ala Ala Arg Asp Leu Val Ser Lys Glu Gly Phe Arg Arg Ala Arg His
265 270 275 280

GTG GTG GGG GAG ATT CGG CGC ACG GCC CAG GCA GCG GCC GCC CTG AGA
916
Val Val Gly Glu Ile Arg Arg Thr Ala Gln Ala Ala Ala Ala Leu Arg
285 290 295

CGT GGC GAC TAC AGA GCC TTT GGC CGC CTC ATG GTG GAG AGC CAC CGC
964
Arg Gly Asp Tyr Arg Ala Phe Gly Arg Leu Met Val Glu Ser His Arg
300 305 310

TCA CTC AGA GAC GAC TAT GAG GTG AGC TGC CCA GAG CTG GAC CAG CTG
1012
Ser Leu Arg Asp Asp Tyr Glu Val Ser Cys Pro Glu Leu Asp Gln Leu
315 320 325

GTG GAG GCT GCG CTT GCT GTG CCT GGG GTT TAT GGC AGC CGC ATG ACG
1060
Val Glu Ala Ala Leu Ala Val Pro Gly Val Tyr Gly Ser Arg Met Thr
330 335 340

GGC GGT GGC TTC GGT GGC TGC ACG GTG ACA CTG CTG GAG GCC TCC GCT
1108
Gly Gly Gly Phe Gly Gly Cys Thr Val Thr Leu Leu Glu Ala Ser Ala

345 350 355 360

GCT CCC CAC GCC ATG CGG CAC ATC CAG GAG CAC TAC GGC GGG ACT GCC
1156
Ala Pro His Ala Met Arg His Ile Gln Glu His Tyr Gly Gly Thr Ala
365 370 375

ACC TTC TAC CTC TCT CAA GCA GCC GAT GGA GCC AAG GTG CTG TGC TTG
1204
Thr Phe Tyr Leu Ser Gln Ala Ala Asp Gly Ala Lys Val Leu Cys Leu
380 385 390

TGAGGCACCC CCAGGACAGC ACACGGTGAG GGTGCGGGGC CTGCAGGCCA GTCCCACGGC
1264

TCTGTGCCCG GTGCCATCTT CCATATCCGG GTGCTCAATA AACTTGTGCC TCCAATGTGG
1324

AAAAAAAAAA AAAAAAAAAC TCGAG
1349

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1349 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 29..1204

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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                               Met Ala Ala Leu Arg Gln Pro Gln
                                 1               5

GTC GCG GAG CTG CTG GCC GAG GCC CGG CGA GCC TTC CGG GAG GAG TTC
100
Val Ala Glu Leu Leu Ala Glu Ala Arg Arg Ala Phe Arg Glu Glu Phe
     10                  15                  20

GGG GCC GAG CCC GAG CTG GCC ATG TCA GCG CCG GGC CGC GTC AAC CTC
148
Gly Ala Glu Pro Glu Leu Ala Met Ser Ala Pro Gly Arg Val Asn Leu
 25                  30                  35                  40

ATC GGG GAA CAC ACG GAC TAC AAC CAG GGC CTG GTG CTG CCT ATG GCT
196
Ile Gly Glu His Thr Asp Tyr Asn Gln Gly Leu Val Leu Pro Met Ala
                 45                  50                  55

CTG GAG CTC ATG ACG GTG CTG GTG GGC AGC CCC CGC AAG GAT GGG CTG
244
Leu Glu Leu Met Thr Val Leu Val Gly Ser Pro Arg Lys Asp Gly Leu
             60                  65                  70

GTG TCT CTC CTC ACC ACC TCT GAG GGT GCC GAT GAG CCC CAG CGG CTG
292
Val Ser Leu Leu Thr Thr Ser Glu Gly Ala Asp Glu Pro Gln Arg Leu
         75                  80                  85

CAG TTT CCA CTG CCC ACA GCC CAG CGC TCG CTG GAG CCT GGG ACT CCT
340
Gln Phe Pro Leu Pro Thr Ala Gln Arg Ser Leu Glu Pro Gly Thr Pro
     90                  95                  100

CGG TGG GCC AAC TAT GTC AAG GGA GTG ATT CAG TAC TAC CCA GCT GCC
388
Arg Trp Ala Asn Tyr Val Lys Gly Val Ile Gln Tyr Tyr Pro Ala Ala
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105 110 115 120

CCC CTC CCT GGC TTC AGT GCA GTG GTC AGC TCA GTG CCC CTG GGG
436
Pro Leu Pro Gly Phe Ser Ala Val Val Ser Ser Val Pro Leu Gly
125 130 135

GGT GGC CTG TCC AGC TCA GCA TCC TTG GAA GTG GCC ACG TAC ACC TTC
484
Gly Gly Leu Ser Ser Ser Ala Ser Leu Glu Val Ala Thr Tyr Thr Phe
140 145 150

CTC CAG CAG CTC TGT CCA GAC TCG GGC ACA ATA GCT GCC CGC GCC CAG
532
Leu Gln Gln Leu Cys Pro Asp Ser Gly Thr Ile Ala Ala Arg Ala Gln
155 160 165

GTG TGT CAG CAG GCC GAG CAC AGC TTC GCA GGG ATG CCC TGT GGC ATC
580
Val Cys Gln Gln Ala Glu His Ser Phe Ala Gly Met Pro Cys Gly Ile
170 175 180

ATG GAC CAG TTC ATC TCA CTT ATG GGA CAG AAA GGC CAC GCG CTG CTC
628
Met Asp Gln Phe Ile Ser Leu Met Gly Gln Lys Gly His Ala Leu Leu
185 190 195 200

ATT GAC TGC AGG TCC TTG GAG ACC AGC CTG GTG CCA CTC TCG GAC CCC
676
Ile Asp Cys Arg Ser Leu Glu Thr Ser Leu Val Pro Leu Ser Asp Pro
205 210 215

AAG CTG GCC GTG CTC ATC ACC AAC TCT AAT GTC CGC CAC TCC CTG GCC
724
Lys Leu Ala Val Leu Ile Thr Asn Ser Asn Val Arg His Ser Leu Ala
220 225 230

TCC AGC GAG TAC CCT GTG CGG CGG CGC CAA TGT GAA GAA GTG GCC CGG
772
Ser Ser Glu Tyr Pro Val Arg Arg Arg Gln Cys Glu Glu Val Ala Arg

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          235                 240                 245

GCG CTG GGC AAG GAA AGC CTC CGG GAG GTA CAA CTG GAA GAG CTA GAG
820
Ala Leu Gly Lys Glu Ser Leu Arg Glu Val Gln Leu Glu Glu Leu Glu
    250                 255                 260

GCT GCC AGG GAC CTG GTG AGC AAA GAG GGC TTC CGG CGG GCC CGG CAC
868
Ala Ala Arg Asp Leu Val Ser Lys Glu Gly Phe Arg Arg Ala Arg His
265                 270                 275                 280

GTG GTG GGG GAG ATT CGG CGC ACG GCC CAG GCA GCG GCC GCC CTG AGA
916
Val Val Gly Glu Ile Arg Arg Thr Ala Gln Ala Ala Ala Ala Leu Arg
                285                 290                 295

CGT GGC GAC TAC AGA GCC TTT GGC CGC CTC ATG GTG GAG AGC CAC CGC
964
Arg Gly Asp Tyr Arg Ala Phe Gly Arg Leu Met Val Glu Ser His Arg
            300                 305                 310

TCA CTC AGA GAC GAC TAT GAG GTG AGC TGC CCA GAG CTG GAC CAG CTG
1012
Ser Leu Arg Asp Asp Tyr Glu Val Ser Cys Pro Glu Leu Asp Gln Leu
        315                 320                 325

GTG GAG GCT GCG CTT GCT GTG CCT GGG GTT TAT GGC AGC CGC ATG ACG
1060
Val Glu Ala Ala Leu Ala Val Pro Gly Val Tyr Gly Ser Arg Met Thr
    330                 335                 340

GGC GGT GGC TTC GGT GGC TGC ACG GTG ACA CTG CTG GAG GCC TCC GCT
1108
Gly Gly Gly Phe Gly Gly Cys Thr Val Thr Leu Leu Glu Ala Ser Ala
345                 350                 355                 360

GCT CCC CAC GCC ATG CGG CAC ATC CAG GAG CAC TAC GGC GGG ACT GCC
1156
Ala Pro His Ala Met Arg His Ile Gln Glu His Tyr Gly Gly Thr Ala
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365 370 375

ACC TTC TAC CTC TCT CAA GCA GCC GAT GGA GCC AAG GTG CTG TGC TTG
1204
Thr Phe Tyr Leu Ser Gln Ala Ala Asp Gly Ala Lys Val Leu Cys Leu
380 385 390

TGAGGCACCC CCAGGACAGC ACACGGTGAG GGTGCGGGGC CTGCAGGCCA GTCCCACGGC
1264

TCTGTGCCCG GTGCCATCTT CCATATCCGG GTGCTCAATA AACTTGTGCC TCCAATGTGG
1324

AAAAAAAAAA AAAAAAAAAC TCGAG
1349

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1349 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 29..265

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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Met Ala Ala Leu Arg Gln Pro Gln
1 5

GTC GCG GAG CTG CTG GCC GAG GCC CGG CGA GCC TTC CGG GAG GAG TTC
100
Val Ala Glu Leu Leu Ala Glu Ala Arg Arg Ala Phe Arg Glu Glu Phe
10 15 20

GGG GCC GAG CCC GAG CTG GCC GTG TCA GCG CCG GGC CGC GTC AAC CTC
148
Gly Ala Glu Pro Glu Leu Ala Val Ser Ala Pro Gly Arg Val Asn Leu
25 30 35 40

ATC GGG GAA CAC ACG GAC TAC AAC CAG GGC CTG GTG CTG CCT ATG GCT
196
Ile Gly Glu His Thr Asp Tyr Asn Gln Gly Leu Val Leu Pro Met Ala
45 50 55

CTG GAG CTC ATG ACG GTG CTG GTG GGC AGC CCC CGC AAG GAT GGG CTG
244
Leu Glu Leu Met Thr Val Leu Val Gly Ser Pro Arg Lys Asp Gly Leu
60 65 70

GTG TCT CTC CTC ACC ACC TCT TAGGGTGCCG ATGAGCCCCA GCGGCTGCAG
295
Val Ser Leu Leu Thr Thr Ser
75

TTTCCACTGC CCACAGCCCA GCGCTCGCTG GAGCCTGGGA CTCCTCGGTG GGCCAACTAT
355

GTCAAGGGAG TGATTCAGTA CTACCCAGCT GCCCCCCTCC CTGGCTTCAG TGCAGTGGTG
415

GTCAGCTCAG TGCCCCTGGG GGGTGGCCTG TCCAGCTCAG CATCCTTGGA AGTGGCCACG
475

TACACCTTCC TCCAGCAGCT CTGTCCAGAC TCGGGCACAA TAGCTGCCCG CGCCCAGGTG
535

TGTCAGCAGG CCGAGCACAG CTTCGCAGGG ATGCCCTGTG GCATCATGGA CCAGTTCATC
595

TCACTTATGG GACAGAAAGG CCACGCGCTG CTCATTGACT GCAGGTCCTT GGAGACCAGC
655

CTGGTGCCAC TCTCGGACCC CAAGCTGGCC GTGCTCATCA CCAACTCTAA TGTCCGCCAC
715

TCCCTGGCCT CCAGCGAGTA CCCTGTGCGG CGGCGCCAAT GTGAAGAAGT GGCCCGGGCG
775

CTGGGCAAGG AAAGCCTCCG GGAGGTACAA CTGGAAGAGC TAGAGGCTGC CAGGGACCTG
835

GTGAGCAAAG AGGGCTTCCG GCGGGCCCGG CACGTGGTGG GGGAGATTCG GCGCACGGCC
895

CAGGCAGCGG CCGCCCTGAG ACGTGGCGAC TACAGAGCCT TTGGCCGCCT CATGGTGGAG
955

AGCCACCGCT CACTCAGAGA CGACTATGAG GTGAGCTGCC CAGAGCTGGA CCAGCTGGTG
1015

GAGGCTGCGC TTGCTGTGCC TGGGGTTTAT GGCAGCCGCA TGACGGGCGG TGGCTTCGGT
1075

GGCTGCACGG TGACACTGCT GGAGGCCTCC GCTGCTCCCC ACGCCATGCG GCACATCCAG
1135

GAGCACTACG GCGGGACTGC CACCTTCTAC CTCTCTCAAG CAGCCGATGG AGCCAAGGTG
1195

CTGTGCTTGT GAGGCACCCC CAGGACAGCA CACGGTGAGG GTGCGGGGCC TGCAGGCCAG
1255

TCCCACGGCT CTGTGCCCGG TGCCATCTTC CATATCCGGG TGCTCAATAA ACTTGTGCCT
1315

CCAATGTGGA AAAAAAAAAA AAAAAAAACT CGAG
1349

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7676 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCGAGCATCC CGCGCCGACG GGTCTGTGCC GGAGCAGCTG TGCAGAGCTG CAGGCGCGCG 60

TCATGGCTGC TTTGAGACAG CCCCAGGTCG CGGAGCTGCT GGCCGAGGCC CGGCGAGCCT 120

TCCGGGAGGA GTTCGGGGCC GAGCCCGAGC TGGCCGTGTC AGCGCCGGGC CGCGTCAACC 180

TCATCGGGGA ACACACGGAC TACAACCAGG GCCTGGTGCT GCCTATGGTG AGGGGCTGCA 240

CGGGGAGCCC CTAGCCCGCC GCCGCCTGTC CCGGTCGCCG AGGAGGGCGG GCCTCGGGGA 300

CGCTGGGGGC GAGTTCTTCC CGCGGGAGAT GTGGGGCGGG CAGCTGCGCC TGGAGCACCG 360

GTGCACGGAA GAGTCCCCGG GACAGGCTGT TCCCCACGTT GGAAGGGAGG AAGCGAAGAA 420

GTGGTCCCCA GAGGGTGCGC GGCCGCCTCT TGGCTCAAGC CCGCCCTCTG GGGGCTGGGG 480

CTCCTCGCCT TCAACCTGGG AGCATGTTCC CCTTAAACTG TGAGGCCCTG TGTGCCACGC
540

AGAAGGGGAC ACTCCGCGCC TCCGGCCACC GTGGGGCCCC AACCGCAGAC CTGGGCGAAC
600

GTAGCCTTCT GGCCCAGCCC GTTCAATTTA CAGAGGAGGA AACTGAGGCC TAGAGAGGCC
660

CAGTGAACTG CTGGAGGTCA CACAGCAGGT TCTTGGCGGG GCTGCGACTT GGGAGTGAGG
720

ACTCCCAGCT TTCAGCGGGG GGCGCTTTCC GCCCCATCTG CAGCTTGGGG AGTGCACAGG
780

TACAGGATGT CCAGAGCCAC CCAAAATGTA AAGGCTTTGG AGCTCCAGTG ATCTGTTTTC
840

CCTTTGGGCT AAGCTCTCCC CCCTTGCCCC ACAGCTCAGG GCAGAGTCCA GGTCTGTGCT
900

CCAGCTGCAG CCGCCCCGCC CCTGAAGACC TAAGGGGGCA GGGCTCAAGC CCCCAAGGTC
960

AGCTGGCCCT CAGGATCTTC CCTGCGACGC TGAACCTGGA GGTTCAGAAC CTGATGACTG
1020

TGGAGGCATC AGAACCTCGG CTGGAGGCAG TGTCATTGGA GAGGCTTACT CCAGCTGGCG
1080

GAAGCCTCAC GTACTGCTTG TCTCTCCTGC CAGGCTCTGG AGCTCATGAC GGTGCTGGTG
1140

GGCAGCCCCC GCAAGGATGG GCTGGTGTCT CTCCTCACCA CCTCTGAGGG TGCCGATGAG
1200

CCCCAGCGGC TGCAGTTTCC ACTGCCCACA GCCCAGCGCT CGCTGGAGCC TGGGACTCCT
1260

CGGTGGGCCA ACTATGTCAA GGGAGTGATT CAGTACTACC CAGGTATGGG GCCCAGGCCT
1320

GAGCCAAGTC CTCACTGATA CTAGGAGTGC CACCTCACAG CCACAGAGCC CATTCATTTG
1380

TCTGATACAC TGTGGGGAAG GCTTGTAGAG TGGAGCATCC CATTGTACAG ATGAGGAAAC
1440

TGATGCCCCC AGAAGGTCGG GAACTTGCCC TGGGTTTCCC GTGACCTGAT TGGAGGAGCC
1500

AGGATTTGAA CCCCAGCCTT TTTTCCCTCC AGAGCCCTAA ACCAGGAGGA CAATTAGAAG
1560

TGTCCCAGCA ACCTCAGAGG GTGGGAAAAT GGAGGGGAGT GGGTCCCTTG GGCCAGCAGG
1620

TTGGTGGGGT TCTTGACAAT TGAGACACAC ACCTAGAAAC AGTTGCTAGG CCGTTGCTGC
1680

CCTTCCCGCC AGGACACCTG CCCTTCCTGT CCAATCCTCC CAGGCAGCCT CTCTTACCAT
1740

CACCTGTTCT TTCCCCCTGC AGCTGCCCCC CTCCCTGGCT TCAGTGCAGT GGTGGTCAGC
1800

TCAGTGCCCC TGGGGGGTGG CCTGTCCAGC TCAGCATCCT TGGAAGTGGC CACGTACACC
1860

TTCCTCCAGC AGCTCTGTCC AGGTACCAGC TAGGCCCCAG CCCTGACCCA GCCCTCCTTC
1920

CCTGAGGTCT CCAGGTGGTC CCAGCTTCTA CTATGCCTTA TGGAGGGGGT GGCAGGGAAT
1980

CTCCCTGGAG TGTCATTGAA GCCACTGCTG CTTCCACCAG CCCTAGCCTC CCCACCTCAC
2040

CCTGTACTGC AGACTCGGGC ACAATAGCTG CCCGCGCCCA GGTGTGTCAG CAGGCCGAGC
2100

ACAGCTTCGC AGGGATGCCC TGTGGCATCA TGGACCAGTT CATCTCACTT ATGGGACAGA
2160

AAGGCCACGC GCTGCTCATT GACTGCAGGT TGGGCTCGCT CCCCTCGTCC CCTCCCGCCC
2220

TGCACTCAGC AGCTCCTGGG TGGAGTGTGC CCACTGCCTG GCGCAGCAAG CACACGCTTG
2280

GCCTCGTCAT CTCCCCCATT GTAACTCCAC CCCAGGTCCT TGGAGACCAG CCTGGTGCCA
2340

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2400

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2460

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2520

TCCTGGAGGC GGCTGTGCTC CCTGCTGGCG CCTCAGTGTG GCCTTGACCC TGCCTGGGAC
2580

CCCGATCTCC AGGGGCTTCT GCCATGCTCT CCCCAGTCCC TTCAAACACT GCGCACCCAG
2640

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2700

CATGTTTCCA TTGTGGAAAA TGTAGAAAAG TACAAAGTAG AAAATAATAA GCTATAAGGG
2760

CACTACCCAG AGATAGGCAC TGCTGACATT TTCACGTTTC CTTTCAGTAT TTTTCCACAT
2820

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2880

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2940

ACTGCAAACT TGAACTCTTG AGCTCAAGGG ATCCTCCCAG CTCAGCCTTC CAAGTAGCTG
3000

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3060

TGTTGCCCAG GATGATCCTG AACTCTGGCC TCAAGAGGTC CTCCTGCCTT GGGCTCCCAA
3120

AGTGTTGGGA TTATAGGCAT GAGCCACTGC GGCCAGCCCA TTTGCCGTGT TTTTTTTTTG
3180

GACACAGAGT TTCGGTCTTG TCACCCATGC TGGAGTGCAA TGGTGCGATC TCAGCTCACT
3240

GTAACCTCTG CCTCCCGGGT TCAAGTGATT CTCCTGCCTC AGCCTCCCGA GTAGCTGGGA
3300

CTACAGGCGC CCGCCACTAC GCCTGGCACA TTTTTTATAG TTCTAGTAGA GACTGGGGTT
3360

TCACCATGTT GGCCAGGCTG GTCTCAAACG CCTGACCTCA GGTGATCCTC CCGCCTCAGC
3420

CTTCCAAAGT GCTGGGATTA CAGGCGTGAG CCATAGTGCC GGTCTCTTTT TTTTTTTTTT
3480

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3540

ATCTCGGCGT GGCTCTTTTT TTTTTTTTTT CTTTTTTTTT GGGCGAGGTG GAGGCTTGCC
3600

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3660

GGTCCAAATG ATCCTCCTGC CTTAGCTTCC TGAGTAGGTG GGATTACTGG AACCCACCAC
3720

CACGCCCAGC CAATTTTTAT ATTTTTAGTA GAGACGGGGT TTCATGTTGG CCAGGCTGGC
3780

CTCGAACTCC TGACCTCGTG ATCTGCCCGC CTCAGCCTCC CAATGTGCTA GGATTACATG
3840

TGTGAGCCAC TGCACCTGGC CTCCGTGTGG CTCTTTAAAG CTCCACAATA TTTTAGCATT
3900

CAGGTGCTCT GTCATTTACT TAACTATTTT CTGATACACC TCACACTGCG ATTAACTTTC
3960

CTTATTTATC TTTTTTATTA TTTATTTATT TATTTATTTG AGACAGAGTC TTGCTCTGTC
4020

ACCCAGGCTG GAGTGCAGTG GCACGATCTC GGCTCACTGC AACCTCTGCC TCCCAGGTTC
4080

AAGTGATTCT CCTGCCTCAG CCTCCTGAGT AGCTAGGATT AGAGGCATGT GCCACCACAC
4140

CTGGCTAATC TTCGTATTTT TAGCAGAGAT GAGGTTTTAC CATGTTGGTC GGGCTGGTCG
4200

TGAACTCCTG ACCTGGTGAT CTGCCCACCT CAGCCTCCCA AAGTACTGGG ATGACAGGCA
4260

TGAACCACTG TGCCTGGCCA TCTTTTTTAT TTTTTAAAGA GATGGGTTCT GCTAAGTTGC
4320

CCAGGCTGGA CCTGAACTCT TGGGCTCAAG TAATCTTCTC ACCTAGTCTC CTGGGTAGCT
4380

GCAACCAAAG GCACCCGGTT TATCTGCATT CTCTTTTTTT TCTTTGAGAC TGAGTCTTGC
4440

TCTGTAGCCC AGGCTGGAGC GCAGTGGCGT GATCTCGGCT CACTGCAACC TCCGTCTTCA
4500

GGGTTCAAGC AATTCTCCTG CCTCAGCCTC TGGAGTGGCT GGGACTACAG GCGTGTGCCA
4560

CCAGAGCGAG TTAATTTTTT TTTTTTTTTG TATTTTTAGT GGACACTGGG TTTCACTATA
4620

TTGGCCAGGC TGGTCTTGGA CTCCTGACCT CAAGTGATCC GCCTGCCTTG GCCTCCCAAA
4680

GTGCTGGGAT TACAGGCACA GGCGTGAGCC ACTACACCTG GCCTATCTGC ATTCTCTTAA
4740

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4800

TAGGCTGGGT GCGGTGGCTC ACGTCTGTGC CTGTAATCCC AGTACTTTAG GAGGCCAAGG
4860

TGGGCAGATT CATTGAGCTC AGGAATTCGA GACCAGCCTG GGCAACATGG CAAAACCCCA
4920

TCTCTAAAGA AATACAAAAA TTAGCCAGGT GTGGTGGTGT GTGCCTGTAG TCCTAGCTAC
4980

TTAGGAGGCT GGGGTGGGAG GATCAATTGA GCCCAGGAGG TTGAGACTGC AGTGAGCTGT
5040

GATTGCACCA TGGCACTCCA GCCTGGGCCT CAAAGTGAGA TCCTGTCTCC AAAACAAAAA
5100

AGATACAAGT ATCCTTAAGG CTCCTGCTAC ACATGGCCAG GAAGGTAGTC TATTGGACAG
5160

TTTTAAGGTC ATTATCAATA TTAGCTCATT TAATTCCCTC CAAAACTCTG TAAAGCACAT 5220

TCTGCTACCA TAGTTGTCAT ATTTTTGATG GGGGAATCTA CAGTGAGAGG CAGTGCTGGG 5280

ATCTGAACCC CATCTGGACA GATTAGCTCC AGGGCCCATG CTCTTGACTG GCTGGCCGCG 5340

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ACAGCTCCGA GGGACCTCCC TGTCCTTTTC CTTTGTGAAC TGTGTCACGT TCTCCAGAGC 5460

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GAACAAGTTG GTCCCTCCTC CCCACCCCAG CCTGTCCTTG GCCCTCCTCC AGGTCTCCTT 5580

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TTTCTTTTTT TTTTTTGAGA GGGAGCCTCA CTCTGTCACC CAGGCTGAAG TGCAGTGGCA 5820

TGATCTCCAC TCACTGCAAC CTCCGCCTCC CAGGTTCAAG CAATTCTCCT GCCTCAGCCT 5880

CCTGAGTAGC TGGGATTACA GGCGCCTGCC ACGATGCCCG GCTAACTTTT GTATTTTTAG 5940

TAGAGACGGG GTTTCGCCAT GTTGGCCAGG CTGGTCTCGA GCTCCTGACC TCAGGCAATC
6000

TGCCTGCCTC AGCTTCCCAA AGTGCTGGGA TTACAGGTGT GAGCCACCGC GCCCACCCAT
6060

TCATTTCTCA GTCCTTTGAA TCTACTTGCC CCTCCATCCC GCCATGCCAC CTACCCTAAC
6120

AACCTTCCCC CTTAAACCTG CGGGTTTGGC CGGGCGCAGT ACACTGAGTC AGTACTGGTA
6180

CTGACCCAGG TACCCCTCCA GCCTCAGCTC CAGTCAGATG GGACAGCCTG CTGGTCCCTG
6240

GCTGCTTCTG CCCCCTCTTC TGGAGCCCCA GCCCTGGAGG CTCCATGTGG CTCAGCAGAA
6300

CTTCTTCTCC TCCTGCTCTG TGGTGGCCTC TTGAGGGCAG CACTCACCTT GGAAAGCATG
6360

GAGTGTTTCA ACCCTCACTG CTCCCTGAAG GACCAAGGTG TCCCATTTTA CAGTCGGGGG
6420

AGGAGGCACT GTGATAAAGG GGCTCTTCAG ACCCACGTCT GAGAGAGCCA GGCTGCGCCG
6480

CCCCCGCGGC CTTCCACCCT TCACCGTCCA GCCAGGGCCA CTGCCATCAC CGCCTGCTGG
6540

TCCTCACAGG CGTCGGGGCC CCAGGCAGTG AGAAGGCGGC TGCTGACTCC TCTTTCCTCC
6600

CCAGCTGCCA GGGACCTGGT GAGCAAAGAG GGCTTCCGGC GGGCCCGGCA CGTGGTGGGG
6660

GAGATTCGGC GCACGGCCCA GGCAGCGGCC GCCCTGAGAC GTGGCGACTA CAGAGCCTTT
6720

GGCCGCCTCA TGGTGGAGAG CCACCGCTCA CTCAGGTGAG GCCCTCTGGG CGCCCCGCTC 6780

CTGCCGGGCA CAGGCCGGCC CAGGCCCACC CCTTCAATAT CCTCTCTGCA GAGACGACTA 6840

TGAGGTGAGC TGCCCAGAGC TGGACCAGCT GGTGGAGGCT GCGCTTGCTG TGCCTGGGGT 6900

TTATGGCAGC CGCATGACGG GCGGTGGCTT CGGTGGCTGC ACGGTGACAC TGCTGGAGGC 6960

CTCCGCTGCT CCCCACGCCA TGCGGCACAT CCAGGTGGGC GGGCACCAGG GCCTGGGCGG 7020

GCAGGAGCGG CAGCTTCCCG GGGCCCTGCC ACTCACCCCC AGCCCGCCTC TTACAGGAGC 7080

ACTACGGCGG GACTGCCACC TTCTACCTCT CTCAAGCAGC CGATGGAGCC AAGGTGCTGT 7140

GCTTGTGAGG CACCCCCAGG ACAGCACACG GTGAGGGTGC GGGGCCTGCA GGCCAGTCCC 7200

ACGGCTCTGT GCCCGGTGCC ATCTTCCATA TCCGGGTGCT CAATAAACTT GTGCCTCCAA 7260

TGTGGTACCT GCCTCCTCTA GAGGTGGGTG TATGCTTGGG TGTCAGAGAA TGGGGGATGT 7320

CAGAACCGCT CCCCTACCCT AGGGGAGCAC CTCTCAGGCC CCAGAAGAAT GGGCAAGGCA 7380

GGGCCTAGCA GTAGCAAAAC CATTTATTAA GTGCAGAACA AAGGCTGGGT CCTTGTGCTG 7440

CTCCCAGCTC TTTGGTTACA AATAGGTTTG GGCCCACAGA GGACGGACCT TGCCCCCTTC 7500

ATGCCTCCCA GGAGACACCT AGCCCCTGCT CTGTGCATGC GGGTGGGCTG GGCCCCCAGG
7560

GGTGCAAGGA TGGAGTAGCT GAGGAGGCTC CGGGAGAGGA GTCGGGAGGA CGCCTAGTGG
7620

GACATTGCGG GGGTGGCGCA GGGTGCGGTC AAGTTTGGAA GAAACTGTTG GGTCCA
7676

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AGCCTTCCGG GAGGAGTTCG G
21

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTGGTTGTAG TCCGTGTGTT C
21

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCCAGCAGCT CCGCGACCTG G
21

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCTTCCTCCC TTCCAACGTG G
21

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CCCAGGCTCC AGCGAGCGCT G
21

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ACCTCTGAGG GTGCCGATGA G
21

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CCCACAGCTC AGGGCAGAGT C
21

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGACACTTCT AATTGTCCTC C
21

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GATGAACTGG TCCATGATGC C
21

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AGGGGCACTG AGCTGACCAC C
21

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CACTTCTACA CATTGGCGCC G
21

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CTTCGCAGGG ATGCCCTGTG G
21

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

TCATCACCAA CTCTAATGTC C
21

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TGTCAGCAGT GCCTATCTCT G
21

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

AGCAGCGGAG GCCTCCAGCA G
21

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CCTCACCGTG TGCTGTCCTG G
21

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GGCTGCGCTT GCTGTGCCTG G
21

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CCTCACCGTG TGCTGTCCTG G
21

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CCTCACCGTG TGCTGTCCTG G
21

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GCGGGACTGC CACCTTCTAC C
21

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CTCAATAAAC TTGTGCCTCC A
21

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CGGATATGGA AGATGGCACC GGG
23

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

AGAGCTGCAG GCGCGCGTCA TG
22

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CCGAGCATCC CGCGCCGAC
19

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CAGCTGCCCG CCCCACATCT
20

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule encoding human genomic galactokinase, said nucleic acid molecule selected from the group consisting of:

(a) a nucleic acid molecule comprising the sequence as set forth in SEQ ID NO:7; and

(b) a nucleic acid molecule differing from the nucleic acid molecule of (a) in codon sequence due to the degeneracy of the genetic code.

2. A vector comprising the nucleic acid molecule of claim 1.

3. A recombinant host cell comprising the vector of claim 2.

4. An isolated nucleic acid molecule comprising a DNA sequence that encodes nucleotides 29 to 1204 of SEQ ID NO:5 or nucleotides 29 to 265 of SEQ ID NO:6.

5. A vector comprising the nucleic acid molecule of claim 4.

6. The vector according to claim 5 which is a plasmid.

7. A recombinant host cell comprising the vector of claim 5.

8. A process for preparing a human galactokinase protein comprising culturing the recombinant host cell of claim 7 under conditions promoting expression of said protein and recovery thereof.

9. An isolated protein encoded by the DNA sequence of claim 4.

10. A monoclonal antibody that is specifically reactive with the protein of claim 9.

11. A method for diagnosing conditions associated with human galactokinase deficiency which comprises isolating a serum or tissue sample from an individual; allowing such sample to come in contact with an antibody or antibody fragment

which specifically binds to the human galactokinase protein of claim 9 under conditions such that an antigen-antibody complex is formed between said antibody or antibody fragment and said galactokinase protein; and detecting the presence or absence of said complex.

12. A method for diagnosing conditions associated with human galactokinase deficiency which comprises isolating a nucleic acid sample from an individual; assaying said sample and the DNA sequence, or corresponding RNA sequence, that encodes a human galactokinase gene; and comparing differences between said sample and said DNA (or RNA) that encodes nucleotides 29 to 1204 of SEQ ID NO:4, wherein said differences indicate mutations in the human galactokinase gene.

13. The method of claim 12 wherein said sample is RNA which is subsequently amplified by PCR-RT.

14. The method of claim 13 wherein assaying said sample comprises a restriction endonuclease digestion.

15. The method of claim 14 wherein said restriction endonuclease is Msc I.

16. The method of claim 12 wherein assaying said sample comprises a hybridization assay.

17. The method of claim 16 wherein the hybridization assay is heteroduplex electrophoresis which comprises determining differential mobility of heteroduplex products in polyacrylamide gels, said heteroduplex products are the result of hybridization between the nucleic acid sample and the DNA sequence, or corresponding RNA sequence, that encodes nucleotides 29 to 1204 of SEQ ID NO:4.

18. The method of claim 12 wherein assaying said sample comprises gel electrophoresis of restriction fragment length polymorphisms of said nucleic acid sample and the DNA sequence, or corresponding RNA sequence, that encodes nucleotides 29 to 1204 of SEQ ID NO:4.

19. The method of claim 12 wherein assaying said sample comprises DNA sequencing.

20. A method for diagnosing conditions associated with human galactokinase deficiency which comprises isolating cells from an individual containing genomic DNA and assaying said sample by *in situ* hybridization using the DNA sequence that encodes nucleotides 29 to 1204 of SEQ ID NO:4, nucleotides 29 to 1204 of SEQ ID NO:5, or nucleotides 29 to 265 of SEQ ID NO:6; or a fragment that encodes at least one exon of said sequence; or a fragment containing at least 15 contiguous base pairs of said sequence as a probe.

21. A transgenic non-human mammal capable of expresing in any cell thereof the DNA of claim 4.

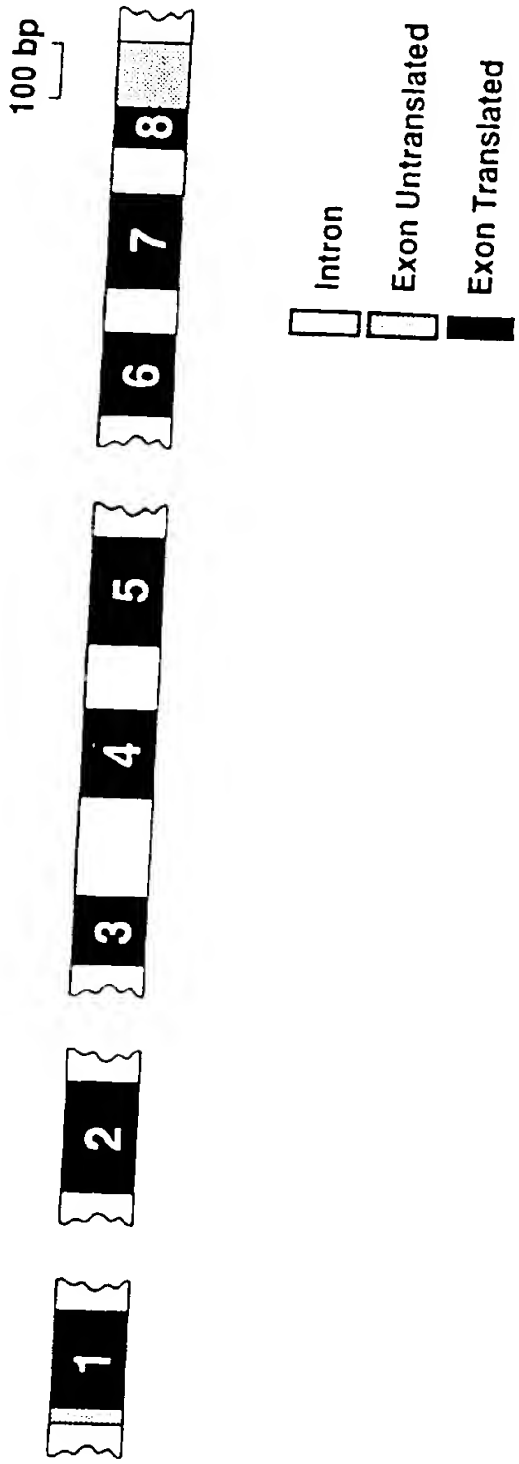

Figure 1

FIGURE 2(a)

```
5'
CCGAGCATCCCGCGCCGACGGGTCTGTGCCGGAGCAGCTGTGCAGAGCTGCAGGCGCGCG    -3
TCATGGCTGCTTTGAGACAGCCCCAGGTCGCGGAGCTGCTGGCCGAGGCCCGGCGAGCCT    58
   M  A  A  L  R  Q  P  Q  V  A  E  L  L  A  E  A  R  R  A

TCCGGGAGGAGTTCGGGGCCGAGCCCGAGCTGGCCGTGTCAGCGCCGGGCCGCGTCAACC   118
F  R  E  E  F  G  A  E  P  E  L  A  V  S  A  P  G  R  V  N

TCATCGGGGAACACACGGACTACAACCAGGGCCTGGTGCTGCCTATGGTGAGGGGCTGCA   178
L  I  G  E  H  T  D  Y  N  Q  G  L  V  L  P  M

CGGGGAGCCCCTAGCCCGCCGCCGCCTGTCCCGGTCGCCGAGGAGGGCGGGCCTCGGGGA   238
CGCTGGGGGCGAGTTCTTCCCGCGGGAGATGTGGGGCGGGCAGCTGCGCCTGGAGCACCG   298
GTGCACGGAAGAGTCCCCGGGACAGGCTGTTCCCCACGTTGGAAGGGAGGAAGCGAAGAA   358
GTGGTCCCCAGAGGGTGCGCGGCCGCCTCTTGGCTCAAGCCCGCCCTCTGGGGGCTGGGG   418
CTCCTCGCCTTCAACCTGGGAGCATGTTCCCCTTAAACTGTGAGGCCCTGTGTGCCACGC   478
AGAAGGGGACACTCCGCGCCTCCGGCCACCGTGGGGCCCCAACCGCAGACCTGGGCGAAC   538
GTAGCCTTCTGGCCCAGCCCGTTCAATTTACAGAGGAGGAAACTGAGGCCTAGAGAGGCC   598
CAGTGAACTGCTGGAGGTCACACAGCAGGTTCTTGGCGGGGCTGCGACTTGGGAGTGAGG   658
ACTCCCAGCTTTCAGCGGGGGGCGCTTTCCGCCCCATCTGCAGCTTGGGGAGTGCACAGG   718
TACAGGATGTCCAGAGCCACCCAAAATGTAAAGGCTTTGGAGCTCCAGTGATCTGTTTTC   778
CCTTTGGGCTAAGCTCTCCCCCCTTGCCCCACAGCTCAGGGCAGAGTCCAGGTCTGTGCT   838
CCAGCTGCAGCCGCCCCGCCCCTGAAGACCTAAGGGGGCAGGGCTCAAGCCCCCAAGGTC   898
AGCTGGCCCTCAGGATCTTCCCTGCGACGCTGAACCTGGAGGTTCAGAACCTGATGACTG   958
TGGAGGCATCAGAACCTCGGCTGGAGGCAGTGTCATTGGAGAGGCTTACTCCAGCTGGCG  1018
GAAGCCTCACGTACTGCTTGTCTCTCCTGCCAGGCTCTGGAGCTCATGACGGTGCTGGTG  1078
                                  A  L  E  L  M  T  V  L  V

GGCAGCCCCCGCAAGGATGGGCTGGTGTCTCTCCTCACCACCTCTGAGGGTGCCGATGAG  1138
 G  S  P  R  K  D  G  L  V  S  L  L  T  T  S  E  G  A  D  E

CCCCAGCGGCTGCAGTTTCCACTGCCCACAGCCCAGCGCTCGCTGGAGCCTGGGACTCCT  1198
 P  Q  R  L  Q  F  P  L  P  T  A  Q  R  S  L  E  P  G  T  P

CGGTGGGCCAACTATGTCAAGGGAGTGATTCAGTACTACCCAGGTATGGGGCCCAGGCCT  1258
 R  W  A  N  Y  V  K  G  V  I  Q  Y  Y  P

GAGCCAAGTCCTCACTGATACTAGGAGTGCCACCTCACAGCCACAGAGCCCATTCATTTG  1318
TCTGATACACTGTGGGGAAGGCTTGTAGAGTGGAGCATCCCATTGTACAGATGAGGAAAC  1378
TGATGCCCCCCAGAAGGTCGGGAACTTGCCCTGGGTTTCCCGTGACCTGATTGGAGGAGCC  1438
AGGATTTGAACCCCAGCCTTTTTTCCCTCCAGAGCCCTAAACCAGGAGGACAATTAGAAG  1489
TGTCCCAGCAACCTCAGAGGGTGGGAAAATGGAGGGGAGTGGGTCCCTTGGGCCAGCAGG  1558
TTGGTGGGGTTCTTGACAATTGAGACACACACCTAGAAACAGTTGCTAGGCCGTTGCTGC  1618
CCTTCCCGCCAGGACACCTGCCCTTCCTGTCCAATCCTCCCAGGCAGCCTCTCTTACCAT  1678
CACCTGTTCTTTCCCCCTGCAGCTGCCCCCCTCCCTGGCTTCAGTGCAGTGGTGGTCAGC  1738
                        A  A  P  L  P  G  F  S  A  V  V  V  S

TCAGTGCCCCTGGGGGGTGGCCTGTCCAGCTCAGCATCCTTGGAAGTGGCCACGTACACC  1798
 S  V  P  L  G  G  G  L  S  S  S  A  S  L  E  V  A  T  Y  T

TTCCTCCAGCAGCTCTGTCCAGGTACCAGCTAGGCCCCAGCCCTGACCCAGCCCTCCTTC  1858
 F  L  Q  Q  L  C  P

CCTGAGGTCTCCAGGTGGTCCCAGCTTCTACTATGCCTTATGGAGGGGGTGGCAGGGAAT  1918
CTCCCTGGAGTGTCATTGAAGCCACTGCTGCTTCCACCAGCCCTAGCCTCCCCACCTCAC  1978
CCTGTACTGCAGACTCGGGCACAATAGCTGCCCGCGCCCAGGTGTGTCAGCAGGCCGAGC  2038
            D  S  G  T  I  A  A  R  A  Q  V  C  Q  Q  A  E

ACAGCTTCGCAGGGATGCCCTGTGGCATCATGGACCAGTTCATCTCACTTATGGGACAGA  2098
H  S  F  A  G  M  P  C  G  I  M  D  Q  F  I  S  L  M  G  Q

AAGGCCACGCGCTGCTCATTGACTGCAGGTTGGGCTCGCTCCCCTCGTCCCCTCCCGCCC  2158
K  G  H  A  L  L  I  D  C  R
```

FIGURE 2(b)

```
TGCACTCAGCAGCTCCTGGGTGGAGTGTGCCCACTGCCTGGCGCAGCAAGCACACGCTTG 2218
GCCTCGTCATCTCCCCCATTGTAACTCCACCCCAGGTCCTTGGAGACCAGCCTGGTGCCA 2278
                                    S  L  E  T  S  L  V  P

CTCTCGGACCCCAAGCTGGCCGTGCTCATCACCAACTCTAATGTCCGCCACTCCCTGGCC 2338
 L  S  D  P  K  L  A  V  L  I  T  N  S  N  V  R  H  S  L  A

TCCAGCGAGTACCCTGTGCGGCGGCGCCAATGTGAAGAAGTGGCCCGGGCGCTGGGCAAG 2398
 S  S  E  Y  P  V  R  R  R  Q  C  E  E  V  A  R  A  L  G  K

GAAAGCCTCCGGGAGGTACAACTGGAAGAGCTAGAGGGTGAGAACTGCCAGGGTGCTCTA 2458
 E  S  L  R  E  V  Q  L  E  E  L  E

TCCTGGAGGCGGCTGTGCTCCCTGCTGGCGCCTCAGTGTGGCCTTGACCCTGCCTGGGAC 2518
CCCGATCTCCAGGGGCTTCTGCCATGCTCTCCCCAGTCCCTTCAAACACTGCGCACCCAG 2578
GGTTCCAATCTCAGCAGGGGTGCTTGAAATCCTAAAATGGTCTTATCTAATCAGAAAAAT 2638
CATGTTTCCATTGTGGAAAATGTAGAAAAGTACAAAGTAGAAAATAATAAGCTATAAGGG 2698
CACTACCCAGAGATAGGCACTGCTGACATTTTCACGTTTCCTTTCAGTATTTTTCCACAT 2758
CTGTCTTCAAAGCTGAGTATATGTAATATATCATCACTTTCCCCCCCCACCCCCTTTTTT 2818
TTAAGAGGCAGGGTCTCATTCTGTTGCCCAAGCTGGAGTGTAGTGGTGTGATCATAGCTT 2878
ACTGCAAACTTGAACTCTTGAGCTCAAGGGATCCTCCCAGCTCAGCCTTCCAAGTAGCTG 2938
AGATTACAGGTGTGCCACCATGCCCGGCTAATTTTTATCTTCGTAAAGACGGCCTTGTAG 2998
TGTTGCCCAGGATGATCCTGAACTCTGGCCTCAAGAGGTCCTCCTGCCTTGGGCTCCCAA 3058
AGTGTTGGGATTATAGGCATGAGCCACTGCGGCCAGCCCATTTGCCGTGTTTTTTTTTTG 3118
GACACAGAGTTTCGGTCTTGTCACCCATGCTGGAGTGCAATGGTGCGATCTCAGCTCACT 3178
GTAACCTCTGCCTCCCGGGTTCAAGTGATTCTCCTGCCTCAGCCTCCCGAGTAGCTGGGA 3238
CTACAGGCGCCCGCCACTACGCCTGGCACATTTTTTATAGTTCTAGTAGAGACTGGGGTT 3298
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CTTCCAAAGTGCTGGGATTACAGGCGTGAGCCATAGTGCCGGTCTCTTTTTTTTTTTTTT 3418
TTAAACTAAACATAATCTCAGAACCCAGAACCCTATCTTATCTTATGCCATGAAAGGCAT 3478
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CTGTTGCCCAGGCTGGAGTGCAGCGGCGCAATCTCGGTTCACTGCATCCTCCACCTCCTG 3598
GGTCCAAATGATCCTCCTGCCTTAGCTTCCTGAGTAGGTGGGATTACTGGAACCCACCAC 3658
CACGCCCAGCCAATTTTTATATTTTTAGTAGAGACGGGGTTTCATGTTGGCCAGGCTGGC 3718
CTCGAACTCCTGACCTCGTGATCTGCCCGCCTCAGCCTCCCAATGTGCTAGGATTACATG 3778
TGTGAGCCACTGCACCTGGCCTCCGTGTGGCTCTTTAAAGCTCCACAATATTTTAGCATT 3838
CAGGTGCTCTGTCATTTACTTAACTATTTTCTGATACACCTCACACTGCGATTAACTTTC 3898
CTTATTTATCTTTTTTATTATTTATTTATTTATTTATTTGAGACAGAGTCTTGCTCTGTC 3958
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AAGTGATTCTCCTGCCTCAGCCTCCTGAGTAGCTAGGATTAGAGGCATGTGCCACCACAC 4078
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TGAACTCCTGACCTGGTGATCTGCCCACCTCAGCCTCCCAAAGTACTGGGATGACAGGCA 4198
TGAACCACTGTGCCTGGCCATCTTTTTTTATTTTTTAAAGAGATGGGTTCTGCTAAGTTGC 4258
CCAGGCTGGACCTGAACTCTTGGGCTCAAGTAATCTTCTCACCTAGTCTCCTGGGTAGCT 4318
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GTGCTGGGATTACAGGCACAGGCGTGAGCCACTACACCTGGCCTATCTGCATTCTCTTAA 4678
TAGTTTCTTAGAAATGGATTCTTAGGAGTAGGATTACAGAGTCAAGAGACACAAGTTTTG 4738
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AGGGCTCAGACCTGCCCTGCCTGCTCTGTGCAGATGCCCTTGGCCAAGGTTTTCACACTG 5458
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```
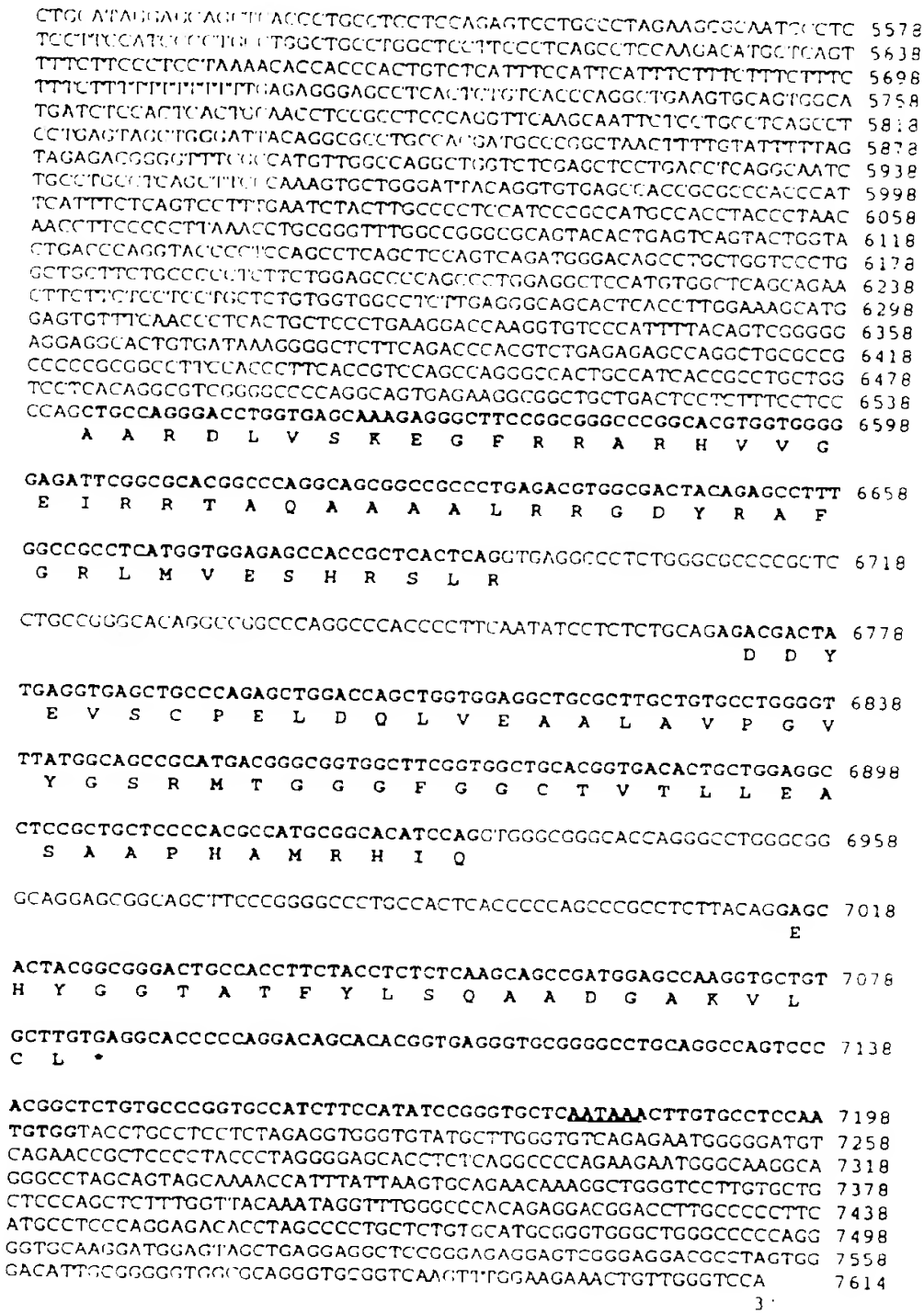

FIGURE 2(c)



SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/06743

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 5/10, 9/12, 15/54, 15/63, 15/85; C12Q 1/00, 1/68; C07K 16/40
US CL :435/6, 7.1, 194, 240.1, 320.1, 536/23.2, 530/388.26,
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.1, 194, 240.1, 320.1, 536/23.2; 530/388.26;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Computer Search - CA, APS, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	Biochimica Biophysica Acta, Volume 831, issued 1985, D. Stambolian, et. al., "Purification Of Human Galactokinase And Evidence For Its Existence As A Monomer Form", Pages 306-312, see entire document.'	1-21
A	Proc. Natl. Acad. Sci., USA, Volume 89, issued November 1992, R. T. Lee, et. al., "Cloning Of A Human Galactokinase Gene (GK2) On Chromosome 15 By Complementation In Yeast", pages 10,887-10,891, see entire document	1-21
A	Nucl. Acids Res., Volume 13, No. 6, issued 1985, C. Debouck, et. al., "Structure Of The Galactokinase Gene Of Escherichia Coli, The Last (?) Gene Of The Gal Operon", pages 1841-1853, see entire document.	1-21

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 03 AUGUST 1995	Date of mailing of the international search report 31 AUG 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer CHARLES PATTERSON Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	Mol. Microbiol., Volume 10, No. 2, issued 1993, P. Glaser, et. al., "Bacillus Subtilis Genome Project: Cloning And Sequencing Of The 97 kb Region From 325 degrees to 333 degrees", pages 371-384, see entire document.	1-21
A	J. Bacteriol., Volume 172, No. 8, issued August 1990, H. H. Houng, et. al., "Molecular Cloning And Physical And Functional Characterization Of The Salmonella Typhimurium and Salmonella Typhi Galactose Utilization Operons", pages 4392-4398, see entire document	1-21

INTERNATIONAL SEARCH REPORT

national application No
PCT/US95/06743

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons

1 ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely

2 ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows

Please See Extra Sheet.

1 ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4 ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1))(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/06743

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claims 1-8 and 21, drawn to nucleic acid, a vector containing the nucleic acid, a host cell containing the vector and a method of use.

Group II, claim 9, drawn to human galactokinase.

Group III, claims 10-11, drawn to an antibody and a method of use.

Group IV, claims 12-20, drawn to a method of diagnosing conditions associated with human galactokinase deficiency using a nucleic acid.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The nucleic acid, vector, host cell and method of use of Group I involve separate and distinct chemical compounds from the enzyme of Group II and antibody of Group III, and therefore Groups I-III do not share a special technical feature. The method of use of the nucleic acid in Group I, namely to prepare a galactokinase protein, is a separate and distinct use from the use of the nucleic acid to diagnose galactokinase deficiencies of Group IV and therefore Groups I and IV do not share a special technical feature. Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single general inventive concept.